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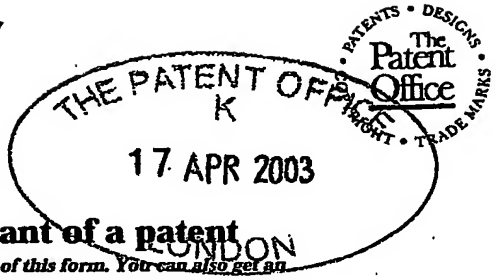

19APR03 E801278-1 002246
P01/7700 0.00-0308988.5

Request for grant of a patent

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1. Your reference — P015404GB CYK

2. Patent application number *(The Patent Office will fill in this part)* — 0308988.5 — 17 APR 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

National University of Singapore
10 Kent Ridge Crescent
Singapore 119260

Patents ADP number *(if you know it)* 70562110001

If the applicant is a corporate body, give the country/state of its incorporation — Singapore

AND Agency for Science, Technology and Research (A*STAR)
10 Science Park Road
#01/01-03 The Alpha
Singapore Science Park 2
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8612947001

Singapore

4. Title of the invention — Molecule

5. Name of your agent *(if you have one)* — D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

21 New Fetter Lane
London
EC4A 1DA

Patents ADP number *(if you know it)* — 59006

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

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b) there is an inventor who is not named as an applicant, or
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11. I/We request the grant of a patent on the basis of this application.

Signature D Young & Co. Date 17 April 2003

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom — Chong-Yee Khoo — 023 8071 9500

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MOLECULE

FIELD

The present invention relates to the fields of microbiology. It also relates to the fields of medicine, especially therapy and diagnosis.

BACKGROUND

Some microorganisms are capable of acting as immunomodulating agents, such as *Mycobacterium smegmatis* used in Freund's complete adjuvant and OK432 from *Streptococcus pygens* as the anti-tumor potentiator. Many polysaccharide immunomodulating agents have also been detected and isolated from *Basidiomycetes* class of fungi, such as lentinan, schizophyllan, TML and SF AI. A novel family of fungal immunomodulatory proteins has been isolated from the edible mushrooms, such as Vvo from *Volvariella volvacea* (grass mushroom), LZ-S from *Ganoderma lucidum* (Ling-Zhi), Gts from *Ganoderma tsugae* (songshan lingzhi), and Fve from *Flammulina velutipes* (golden needle mushroom).

Although the therapeutic value of a number of mushrooms has been documented, the active components that confer such therapeutic effects are not well understood.

Ko et al (Eur. J. Biochem., 228, 244-2419) describes the isolation and purification of a protein known as FIP-*fve* from Golden Needle Mushroom extracts. The authors describe a method of extracting this protein, as well as some biochemical properties of FIP-*fve*. The amino acid sequence of FIP-*fve* is presented. FIP-*fve* is shown to cause proliferation of human peripheral blood lymphocytes, and mice sensitised to BSA are protected against anaphylactic shock by periodic injections of FIP-*fve*. A hind-paw edema test shows that FIP-*fve* inhibits antibody production against antigen 48/80. Finally, the authors show that FIP-*fve* induces expression of IL-2 and IFN-γ in spleen cells from mouse.

An amino acid sequence of FIP-*fve* is found as GenBank accession numbers:S69147 immunomodulatory protein FIP-fve - golden needle mushroom gi|7438667|pir||S69147[7438667] and P80412 IMMUNOMODULATORY PROTEIN FIP-FVE gi|729544|sp|P80412|FVE_FLAVE[729544].

SUMMARY

According to a first aspect of the present invention, we provide an Fve polypeptide comprising at least one biological activity of native Fve protein, and being a fragment, homologue, variant or derivative thereof.

Preferably, the Fve polypeptide comprises an immunomodulatory activity. Preferably, the biological activity is selected from the group consisting of: up-regulation of expression of Th1/Tc1 cytokines, preferably IFN-γ and TNF-α, down-regulation of expression of Th2/Tc2 cytokines, preferably IL-4 and IL-13, up-regulation of expression of T regulatory (Tr) cytokines IL-10 and TGF-β, hemagglutination activity, cell aggregation activity, lymphocyte aggregation activity, lymphoproliferation activity, up-regulation of expression of IL-2, IFN-γ, TNF-α, but not IL-4 in $CD3^+$ T cells, interaction with T and NK cells, adjuvant activity, stimulation of $CD3^+$ $CD16^+$ $CD56^+$ natural killer (NK) T cells and $CD3^+$ $CD8^+$ $CD18^{+\,bright}$ T cells, and up-regulation of allergen specific Th1 immune responses.

Preferably, the polypeptide comprises between 2 to 20 residues of amino acid sequence flanking the glycine residue corresponding to position 28 of Fve.

Preferably, the polypeptide comprises the sequence RGT or the sequence RGD.

Preferably, the polypeptide has a sequence as set out in **Appendix A** or **Appendix B**.

There is provided, according to a second aspect of the present invention, a Fve polypeptide comprising an sequence selected from the group consisting of: Fve R27A, Fve T29A, GST-Fve (wild type), GST-Fve R27A, and GST-Fve T29A, and fragments, homologues, variants and derivatives thereof.

We provide, according to a third aspect of the present invention, a polypeptide comprising a first portion comprising at least a portion of Fve and a second portion comprising at least a portion of an allergen.

Preferably, the allergen comprises an allergen from a mite, preferably from Family *Glycyphagidae* or Family *Pyroglyphidae*, preferably a group 1 allergen (Der p 1, Der f 1, Blo t 1, Eur m1, Lep d 1), a group 2 allergen (Der p 2, Der f 2, Blo t 2, Eur m 2, Lep d 2), a group 5 allergen (Blo t 5, Der p 5, Der f 5, Eur m 5, Lep d 5) a group 15 allergen (Der p 15, Der f 15, Blot 15, Eur m 15, Lep d 15).

Preferably, the Fve polypeptide or a polypeptide is selected from the group consisting of: Blo t 5-Fve, Blo t 5-FveR27A, Blo t 5-FveT29A, GST-Der p 2-FveR27A, GST-Der p 2-FveT29A, Blo t 5-Der p 2-FveR27A, and Blo t 5-Der p 2-FveT29A. More preferably, it comprises Blo t 5-FveT29A, Der p 2-FveT29A, or Blo t 5-Der p 2-FveT29A.

Preferably, the allergen is selected from the group consisting of: tree pollen allergen, Bet v 1 and Bet v 2 from birch tree; grass pollen allergen, Phl p 1 and Phl p 2 from timothy grass; weed pollen allergen, antigen E from ragweed; major feline antigen, Fel d; major fungal allergen, Asp f1, Asp f2, and Asp f3 from *Aspergillus fumigatus*.

As a fourth aspect of the present invention, there is provided a polypeptide comprising a first portion comprising at least a portion of Fve and a second portion comprising at least a portion of a viral antigen selected from the group consisting of: E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; and Tax from HTLV-1.

Preferably, it comprises HCV Core23-FveT29A, or HPV E7-FveT29A.

We also provide a polypeptide comprising a first portion comprising at least a portion of Fve and a second portion comprising at least a portion of a viral antigen selected from the group consisting of antigens from Adenovirus, Parainfluenza 3 virus, Human Immunodeficiency Virus (HIV), Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, and Influenza A, Flu A.

We provide, according to a fifth aspect of the present invention, a polypeptide comprising a first portion comprising at least a portion of Fve and a second portion comprising at least a portion of a tumour-associated antigen selected from the group consisting of: MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, and P15.

Preferably, it comprises MAGE3-FveT29A, MART1-FveT29A or CEA-FveT29A.

The present invention, in a sixth aspect, provides a nucleic acid encoding a Fve polypeptide or a polypeptide according to any preceding statement of invention.

Preferably, the nucleic acid comprises CGT GGT ACC, or a sequence which differs from the above by virtue of the degeneracy of the genetic code and which encodes a sequence RGT.

In a seventh aspect of the present invention, there is provided a nucleic acid comprising a sequence encoding at least a portion of Fve and a sequence encoding at least a portion of an allergen.

Preferably, it comprises Blo t 5-FveT29A, Der p 2-FveT29A, or Blo t 5-Der p 2-FveT29A.

According to an eighth aspect of the present invention, we provide a nucleic acid comprising a sequence encoding at least a portion of Fve and a sequence encoding at least a portion of a viral antigen selected from the group consisting of: E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; and Tax from HTLV-1.

Preferably, it comprises HCV Core23-FveT29A, or HPV E7-FveT29A.

We also provide a nucleic acid comprising a sequence encoding at least a portion of Fve and a sequence encoding at least a portion of a viral antigen selected from the group consisting of antigens from Adenovirus, Parainfluenza 3 virus, Human Immunodeficiency Virus (HIV), Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, and Influenza A, Flu A.

We provide, according to a ninth aspect of the invention, a nucleic acid comprising a sequence encoding at least a portion of Fve and a sequence encoding at least a portion of a tumour associated antigen selected from the group consisting of: MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, and P15.

Preferably, it comprises MAGE3-FveT29A, MART1-FveT29A or CEA-FveT29A.

There is provided, in accordance with a tenth aspect of the present invention, a nucleic acid selected from the group consisting of: Fve R27A, Fve T29A, GST-Fve (wild type), GST-Fve R27A, GST-Fve T29A, Blo t 5-Fve, Blo t 5-FveR27A, Blo t 5-FveT29A, GST-Der p 2-FveR27A, GST-Der p 2-FveT29A, Blo t 5-Der p 2-FveR27A, Blo t 5-Der p 2-FveT29A, and fragments, homologues, variants and derivatives thereof.

As an eleventh aspect of the invention, we provide a vector, preferably an expression vector, comprising a nucleic acid sequence as set out above.

We provide, according to a twelfth aspect of the invention, there is provided DNA vaccine comprising a nucleic acid encoding Fve, a nucleic acid, or a vector as set out above.

According to a thirteenth aspect of the present invention, we provide host cell comprising a nucleic acid encoding Fve, a nucleic acid, or a vector as set out above.

There is provided, according to a fourteenth aspect of the present invention, transgenic non-human organism comprising a nucleic acid encoding Fve, a nucleic acid, or a vector as set out above.

Preferably, the transgenic non-human organism is a bacterium, a yeast, a fungus, a plant or an animal, preferably a mouse.

According to a sixteenth aspect of the present invention, we provide a pharmaceutical composition comprising a polypeptide, a nucleic acid, a vector, a DNA vaccine, or a host cell as set out above, together with a pharmaceutically acceptable carrier or diluent.

According to a seventeenth aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above as an immumodulator.

According to an eighteenth aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above to enhance an immune response in a mammal.

According to a nineteenth aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above to stimulate proliferation of $CD3^+$ $CD8^+$ $CD18^{+\,bright}$ T cells.

According to a twentieth aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above to stimulate proliferation of $CD3^+$ $CD16^+$ $CD56^+$ natural killer (NK) T cells.

According to a twenty first aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above to stimulate production of IL-2, IL-10, TGF-β, IFN-γ or TNF-α in $CD3^+$ cells.

Preferably, production of IL-4 is not stimulated in the $CD3^+$ cells.

According to a twenty second aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above as an adjuvant for a vaccine.

According to a twenty third aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above in a method of treatment or prophylaxis of a disease.

According to a twenty fourth aspect of the present invention, we provide the use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector or host cell as set out above for the preparation of a pharmaceutical composition for the treatment of a disease.

According to a twenty fifth aspect of the present invention, we provide a method of treating an individual suffering from a disease or preventing the occurrence of a disease in an individual, the method comprising administering to the individual a therapeutically or prophylactically effective amount of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition as set out above.

Preferably, the use or method is such that disease comprises an atopic disease or allergy.

Preferably, the allergy is selected from the group consisting of: allergic asthma, a seasonal respiratory allergy, a perennial respiratory allergy, allergic rhinitis, hayfever, nonallergic rhinitis, vasomotor rhinitis, irritant rhinitis, an allergy against grass pollen, weed pollen, tree pollen or animal danders, an allergy associated with allergic asthma and a food allergy.

Preferably, the allergy is to a house dust mite from Family Glyphagidae, preferably *Blomia tropicalis* or from Family Pyroglyphidae, preferably *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*, or to fungi or fungal spores, preferably *Aspergillus fumigatus*.

In an alternative embodiment, the disease comprises a cancer.

According to a twenty seventh aspect of the present invention, we provide the use of a DNA vaccine as described, in a method of treatment or prevention of a cancer, or in a method of suppressing tumour progression.

Preferably, the cancer comprises a T cell lymphoma, melanoma, lung cancer, colon cancer, breast cancer or prostate cancer.

According to a twenty eighth aspect of the present invention, we provide a method of identifying a molecule capable of binding to Fve, the method comprising exposing a native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism according as set out above to a candidate molecule and detecting whether the candidate molecule binds to the native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism.

According to a twenty ninth aspect of the present invention, we provide a method of identifying an agonist or antagonist of an Fve polypeptide, the method comprising: (a) providing a cell or organism; (b) exposing the cell or organism to a native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism as set out above; (c) exposing the cell to a candidate molecule; and (d) detecting an Fve mediated effect.

Preferably, the Fve mediated effect is selected from the biological activities set out above.

Preferably, the method further comprises isolating or synthesising a selected or identified molecule.

According to a thirtieth aspect of the present invention, we provide a molecule identified or selected using such a method.

According to a thirty first aspect of the present invention, we provide a native Fve polypeptide, or an Fve polypeptide in crystalline form.

Preferably, the crystal has the structural coordinates shown in **Appendix C**.

According to a thirty second aspect of the present invention, we provide a model for at least part of Fve made using such a crystal.

According to a thirty third aspect of the present invention, we provide a method of screening for a receptor capable of binding to Fve, or designing a ligand capable of modulating the interaction between Fve and an Fve receptor, comprising the use of such a model.

According to a thirty fourth aspect of the present invention, we provide a computer readable medium having stored thereon the structure of such a crystal or such a model.

According to a thirty fifth aspect of the present invention, we provide a ligand identified by the method set out above.

According to a thirty sixth aspect of the present invention, we provide a use of such a molecule or such a ligand for the treatment or prevention of a disease in an individual.

According to a thirty seventh aspect of the present invention, we provide a pharmaceutical composition comprising such a molecule or such a ligand and optionally a pharmaceutically acceptable carrier, diluent, excipient or adjuvant or any combination thereof.

According to a thirty eighth aspect of the present invention, we provide a method of treating and/or preventing a disease comprising administering such a molecule or such a ligand and/or such a pharmaceutical composition to a mammalian patient.

According to a thirty ninth aspect of the present invention, we provide a method of amplifying a sub-population of cells, the method comprising: (a) obtaining a population of cells from an individual; (b) amplifying $CD3^+$ $CD8^+$ and $CD18^{+\ bright}$ T cells by exposing the population of cells to a native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism as set out above.

Preferably, the method further comprises the step of: (c) isolating the $CD3^+$ $CD8^+$ and $CD18^{+\ bright}$ T cells.

According to a fortieth aspect of the present invention, we provide a method of treating an individual suffering from a disease or preventing the occurrence of a disease in an individual, the method comprising amplifying a $CD3^+$ $CD8^+$ and $CD18^{+\,bright}$ T cell by such a method, and administering the amplified $CD3^+$ $CD8^+$ and $CD18^{+\,bright}$ T cell to an individual.

According to a forty first aspect of the present invention, we provide a combination comprising a first component comprising an immunomodulator and a second component comprising at least a portion of an allergen, a viral antigen or a tumour associated antigen.

Preferably, the first component is separate from the second component. Alternatively, or in addition, the first component may be associated with the second component. Preferably, the combination comprises a fusion protein.

The first component may comprise a native Fve polypeptide, or a polypeptide as set out above. The second component may comprise an allergen selected from the group consisting of: a mite allergen, an mite allergen from Family *Glycyphagidae* or Family *Pyroglyphidae*, a group 1 allergen (Der p 1, Der f 1, Blo t 1, Eur m1, Lep d 1), a group 2 allergen (Der p 2, Der f 2, Blo t 2, Eur m 2, Lep d 2), a group 5 allergen (Blo t 5, Der p 5, Der f 5, Eur m 5, Lep d 5), a group 15 allergen (Der p 15, Der f 15, Blot 15, Eur m 15, Lep d 15), a tree pollen allergen, Bet v 1 and Bet v 2 from birch tree; grass pollen allergen, Phl p 1 and Phl p 2 from timothy grass; weed pollen allergen, antigen E from ragweed; major feline antigen, Fel d; major fungal allergen, Asp f1, Asp f2, and Asp f3 from *Aspergillus fumigatus*.

In preferred embodiments, the second component comprises a viral antigen selected from the group consisting of: E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; and Tax from HTLV-1. Alternatively, or in addition, the second component may comprise a tumour-associated antigen selected from the group consisting of: MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100,

TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, and P15.

We further disclose an immunomodulator-antigen conjugate, preferably an immunomodulator-allergen conjugate, an immunomodulator-tumour associated antigen conjugate or a immunomodulator-viral antigen conjugate, in which the immunomodulator preferably comprises an Fve polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Analysis of purified native Fve by SDS-PAGE and gel filtration chromatography. (a). The native Fve protein purified by cation and anion exchange chromatography is analyzed by Tricine SDS-PAGE. Fve protein gave a single band with an apparent molecular mass of 12.7 kDa. Lane M, molecular mass markers; lane 1, purified native Fve protein. (b) Elution profile of calibration proteins by Superdex 75 chromatography. Peaks, 1. bovine serum albumin (67 kDa); 2. ovalbumin (43 kDa); 3. chymotrypsinogen A (25 kDa); 4. ribonuclease A (13.7 kDa). (c) Purified native Fve formed homodimer at 25.5 kDa.

Figure 2 shows a profile of cytokines and iNOS produced by mouse splenocytes upon stimulation with Fve protein. Mouse spleen cells from Balb/cJ mice are stimulated with 20μg of Fve. The mRNAs of cytokines are analyzed by RT-PCR after culturing for 48 hours. A: A non-stimulated culture as negative controls, B: A culture stimulated with 20μg of Fve.

Figure 3 shows a profile of human cytokines, transcriptional factors, adhesion molecule and anti-apoptotic protein produced by human PBMC upon stimulation with Fve protein. Human PBMC from healthy donor are stimulated with 20μg of Fve. The mRNA expression is analyzed by RT-PCR after culturing for 48 hours. A: A non-stimulated culture as negative control, B: A culture stimulated with 20μg of Fve.

Figure 4. A schematic representation showing the principle of overlap extension PCR for the generation of single amino acid residue substitution (A) and deletion mutagenesis of DNA (B).

Figure 5. A schematic representation of the strategy used to generate mutants. On the basis of the structures predicted by PHD prediction program, eleven deletion mutants and three point mutants of Fve plasmid DNA are generated by PCR-based mutagenesis.

Figure 6. SDS-PAGE analysis of recombinant Fve mutant proteins.

Figure 7. *In vitro* proliferation assay of mouse splenocytes. Mouse splenocytes from Balb/cJ is stimulated with 2.5μg/ml, 5μg/ml, 10μg/ml, and 20μg/ml, respectively, with 13 of Fve mutant proteins for 48 hours. Recombinant GST-Fve is positive control. GST is negative control.

Figure 8. Lymphoproliferation activity of human PBMC at 48 hours. Human PBMC from a healthy donor is stimulated with 2.5μg/ml, 5μg/ml, 10μg /ml, and 20μg /ml, respectively, with eleven of Fve mutant proteins for 48 hours. Recombinant GST-Fve and native Fve are positive control. GST and Blo t 5 are negative control.

Figure 9. Recombinant GST-Fve (wild type) and GST-FveT29 mutant protein showed strong lymphoproliferative activity. Human PBMC from healthy donor are cultured with: (A) no antigen, (B) GST, (C) wild type GST-Fve, (D) GST-FveT29, each protein is used at 20μg /ml. The percentage of $CD3^+$ T lymphocytes is analyzed at day 5 by using flow cytometry.

Figure 10. Increased production of TNF-α, IFN-γ, IL-2 but not IL-4 in $CD3^+$ T lymphocytes after stimulation with native Fve protein. The production of (A) IL-4; (B) IL-2; (C) IFN-γ and (D) TNF-α by human PBMC after stimulation with 20μg /ml of native Fve protein for three days. The data are analyzed by flow cytometry.

Figure 11. Recombinant wild type GST-Fve and mutant GST-FveT29A, but not mutant GST-FveG28A, maintained IFN-γ cytokine production activity. Human PBMC from healthy donor are cultured with 20μg of GST (1); GST-Fve (2); GST-FveR27A (3); GST-FveG28A (4); GST-FveT29A (5). IFN-γ cytokine by T cells is detected at day 3 by staining with anti-CD3 PerCP and anti-IFN-γ FITC specific monoclonal antibody. IFN-γ secretion by small granular lymphocytes and large granular lymphocytes are shown in (a) and (b), respectively. The total amount of IFN-γ production by T cells is the sum of (a) and (b).

Figure 12. Recombinant wild type GST-Fve and mutant GST-FveT29A, but not mutant GST-FveG28A, maintained TNF-α production activity. Human PBMC from healthy donor are cultured with 20μg of GST (1); GST-Fve (2); GST-FveR27A (3); GST-FveG28A (4); GST-FveT29A (5). IFN-γ cytokine by T cells is detected at day 3 by staining with anti-CD3 PerCP and anti- TNF-α FITC specific monoclonal antibody. TNF-α secretion by small granular lymphocytes and large granular lymphocytes are shown in (a) and (b), respectively. The total amount of TNF-α production by T cells is the sum of (a) and (b).

Figure 13. Schematic representation of the experimental design of the *in vivo* study Balb/cJ mice are immunized with Der p 2 in aluminum hydroxide at day 0 and boosted at day 21 by intraperitoneal injection. Treatment with Der p 2 alone or Der p 2 plus Fve is started at day 28 by given 6 subcuteneous injections over 12 days. Mice are challenged with Der p 2 at day 42.

Figure 14. Enhanced anti-Der p 2 IgG2a by adjuvanicity of Fve protein. IgG2a response in mice that are subcutaneously injected six times with Der p 2 alone (close circle), or Der p 2 plus Fve (close square) twenty-eight days after the initial sensitization with Der p 2 in alum. Mice received third intraperitoneal injection with Der p 2 in alum at day 42. Results are shown as mean titers and error bars indicate the standard deviations from the mean titers.

Figure 15. Fve could reduce wheal and erythematic flare formation on skin prick test-positive human subject. Both the left and right hands of the house dust mite allergen sensitized human subject are challenged with saline, histamine, Der p 2, and mixture of Der p 2 and Fve at the separated sites on hands. The diameter sizes of wheel (A) and erythematic flare (B) are measured after 10 minutes incubation time

Figure 16. A schematic representation of the seven fusion proteins of Bt5-Fve (wild type), Bt5-FveR27A, Bt5-FveT29A, Dp2-FveR27A, Dp2-FveT29A, Bt5-Dp2-FveR27A, and Bt5-Dp2-FveT29A.

Figure 17. Expression and purification of recombinant fusion protein Bt5-Fve, Bt5-FveR27A, and GST-Dp2-FveR27A. Lane 1 and 10 are protein marker. Lane 2 to 9 are GST; Blo t 5; Fve; Bt5-Fve; Bt5-FveR27A; Der p 2; Fve; and GST-Bt5, respectively.

Figure 18. Functional characterization of recombinant fusion proteins of Fve and allergen. The morphology of human lymphocytes upon stimulation with three different fusion proteins for three days. All photographs are taken at a magnification of x10 and ×40 with a confocal microscope. 1(a) Control: Non-stimulated (10x10 magnification); 1(b) Control: Non-stimulated (40x10 magnification); 2(a): 20μg of GST 10x10; 2(b): 20μg of GST 40x10; 3(a): 20μg of Blo t 5 10x10; 3(b): 20μg of Blo t 5 40x10; 4(a): 20μg of native Fve 10x10; 4(b): 20μg of native Fve 40x10; 5(a): 20μg of Bt5-Fve 10x10; 5(b): 20μg of Bt5-Fve 40x10; 6(a): 40μg of Bt5-Fve 10x10; 6(b): 40μg of Bt5-Fve 40x10; 7(a) 40μg of Bt5-FveR27A 10x10; 7(b): 40μg of Bt5-FveR27A 40x10; 8(a): 20μg of Der p 2 10x10; 8(b): 20μg of Der p 2 40x10; 9(a): 40μg of GST-Dp2-FveR27A 10x10; 9(b): 40μg of GST-Dp2-FveR27A 40x10. Human lymphocytes maintained aggregation ability upon stimulation with Bt5-Fve (5a, 5b, 6a, 6b) and Bt5-FveR27A (7a, 7b) for 3 days. Native Fve (4a, 4b) is a positive control. Non-stimulated cells (1a, 1b), GST (2a, 2b), Blo t 5 (3a, 3b), and Der p 2 (8a, 8b) are negative controls. The aggregation ability of GST-Dp2-FveR27A is not apparent at day 3 (9a, 9b).

Figure 19. Cell number comparison of human PBMC after 7 days cultured with tested antigens. Human PBMC are cultured with different doses of recombinant allergen and Fve fusion proteins. Non-stimulated cells and cells stimulated with either 20μg of Blo t 5; 20μg of Fve; 20μg of Bt5-Fve; 40μg of Bt5-Fve; 20μg of Bt5-FveR27A; and 40μg of Bt5-FveR27A are shown in Figure 19A. Cells stimulated with 20μg of Der p 2; 20μg of GST-Dp2-FveR27A; and 40μg of GST-Dp2-FveR27A are shown in Figure 19B. The cells are collected and counted at day 7.

Figure 20. The lymphoproliferation activity of human lymphocytes upon stimulation with recombinant fusion protein Bt5-Fve for 72 hours. Human PBMC from a healthy donor is co-cultured with 5μg /ml, 10μg /ml, 20μg /ml, and 40μg /ml, respectively, with fusion protein Bt5-Fve (BFwt). Recombinant GST and Blo t 5 are used as negative controls. Fve is used a positive control.

Figure 21. Bt5Fve fusion protein maintained CD8 T cells polarization activity. Human PBMC are isolated from healthy donar and stimulated with 20μg of GST (b), 20μg of Blo t 5 allergen (c), 20μg of Fve (d), 20μg of Bt5Fve (e), 40μg of Bt5Fve (f), 20μg of Bt5FveR27 (g), and 40μg of Bt5FveR27 (h) for 5 days. Cells without any stimulation served as negative control (a). Cultured cells are stained with CD3-PerCP and CD8-FITC monoclonal antibodies and analyzed with FACSCalibur cytometry.

Figure 22. Fve and allergen-Fve fusion protein are able to induce T helper type 1 and T regulatory immune responses. (A). Fve induced IFN-γ and IL-10 production. Human PBMC from seven individuals are cultured with 20μg of Fve. The production of IFN-γ, IL-4 and IL-10 is assayed by ELISA at day 3. (B). Comparable levels of IFN-γ production are induced by Fve and allergen – Fve fusion protein. Human PBMC are stimulated with Fve, Blot5, Blot5-Fve (wild type) and Blot5-FveR27A (mutant), respectively. The production of IL-4 and IFN-γ is detected by ELISA at day 3 and day 7.

Figure 23. Competitive inhibition assay. Varying concentrations of inhibitors are used to inhibit the binding of human IgE to GST-Blot5 bound to the Elisa plate. The

concentration of different inhibitors ranged from 0.01ng to 10000ng/ml. Results are obtained from serum of a representative allergic subject with high IgE reactivity to house dust mite allergens. GST: Glutathione S-transferase. GF: GST-Fve. GFB: GST-Fve-Blot5. GBF: GST-Blot5-Fve. BF: Blot5-Fve. B: Blo t 5.

Figure 24. Human PBMC stimulated with native Fve protein for five days showed a significant increase in $CD16^+$ and $CD56^+$ cells. The $CD3^+$ cells and $CD16^+ + CD56^+$ cells are analyzed by FACScan after staining with anti-CD3 FITC, anti-CD16 PE and anti-CD56 PE conjugated mouse anti-human specific monoclonal antibody. Cells stimulated with (a) no antigen; (b). 5μg of Der p 2 house dust mite allergen as negative control; (c). 5μg of PHA; (d). 5μg of Fve; (e). 25μg of Fve.

Figure 25. Human PBMC stimulated with Fve protein for ten days showed a significant increase in $CD8^+$ cells. The proportion of $CD4^+$ and $CD8^+$ T cells are analyzed by FACScan after staining with anti-CD4 FITC and anti-CD8 PE conjugated mouse anti-human specific monoclonal antibody. Cells are stimulated with (a). no antigen; (b). 5μg of Der p 2 house dust mite allergen as negative control; (c). 5μg of PHA; (d). 5μg of Fve; (e). 25μg of Fve.

Figure 26. Expanded human $CD3^+CD18^{+Bright}$ T cells subset in human PBMC after stimulation with Fve protein for five days. Human PBMC from healthy donor are cultured alone (a and c) or with 20μg of native Fve protein (b and d) for 5 days. Cells are then analyzed by flow cytometry after staining with anti-CD3 PerCP, anti-CD8 PE and anti-CD18 FITC.

Figure 27. Expanded $CD3^+CD8^{+Bright}CD18^{+Bright}$ T cells in human PBMC after cultured with Fve protein for five days. Human PBMC from healthy donor are cultured alone (a and c) or with 20μg of native Fve protein (b and d) for five days. Cells are analyzed by flow cytometry after staining with anti-CD3 PerCP, anti-CD8 PE and anti-CD18 FITC.

Figure 28. Proportion of *in vivo* BrdU incorporated natural killer (NK) cells from spleen of C57BL/6J naïve mice (a), or mouse received three consecutive subcutaneous injections with 10μg of Fve (b), 50μg of Fve (c), 250μg of Fve (d). Splenocytes are stained with anti-Pan NK PE and anti-BrdU FITC monoclonal antibodies and then analyzed with flow cytometry.

Figure 29. Proportion of *in vivo* BrdU incorporated $CD8^+$ T cells from spleen of C57BL/6J naïve mice (a), or mouse received three consecutive subcutaneous injections with 10μg of Fve (b), 50μg of Fve (c), 250μg of Fve (d). Splenocytes are stained with anti-CD8 PE and anti-BrdU FITC monoclonal antibodies and then analyzed with flow cytometry.

Figure 30. Proportion of *in vivo* BrdU incorporated $CD4^+$ T cells from spleen of C57BL/6J naïve mice (a), or mouse received three consecutive subcutaneous injections with 10μg of Fve (b), 50μg of Fve (c), 250μg of Fve (d). Splenocytes are stained with anti-CD4 PE and anti-BrdU FITC monoclonal antibodies and then analyzed with flow cytometry.

Figure 31. Proportion of *in vivo* BrdU incorporated $CD19^+$ B cells from spleen of C57BL/6J naïve mice (a), or mouse received three consecutive subcutaneous injections with 10μg of Fve (b), 50μg of Fve (c), 250μg of Fve (d). Splenocytes are stained with anti-CD19 PE and anti-BrdU FITC monoclonal antibodies and then analyzed with flow cytometry.

Figure 32. Proportion of *in vivo* BrdU incorporated $CD8^+$ T cells from lymph nodes of C57BL/6J naïve mice (a), or mouse received three consecutive subcutaneous injections with 10μg of Fve (b), 50μg of Fve (c), 250μg of Fve (d). Lymph nodes are stained with anti-CD8 PE and anti-BrdU FITC monoclonal antibodies and then analyzed with flow cytometry.

Figure 33. Proportion of $CD4^+$ and $CD8^+$ T cell subsets from mouse peripheral blood mononuclear cells of Balb/cJ naïve mouse (a), or mouse received seven consecutive subcutaneous injections with 125μg of Fve. Panels (b), (c), (d) represent results for three respective individual mouse. Mouse peripheral blood mononuclear cells are collected in a tube with anti-coagulant. Cells are stained with anti-CD8 PE and anti-CD4 FITC monoclonal antibodies and then analyzed by flow cytometry.

Figure 34. Schematic representative of two mammalian eukaryotic expression vectors. (A) pCI-neo can constitutively express high level of recombinant protein in mammalian cells (Picture adopted from Promega, USA). (B) pDisplay can display recombinant protein to the surface of mammalian cells (Picture adopted from Invitrogen life technologies, USA).

Figure 35. Growth suppression of EL4 solid tumor. C57BL mice are inoculated with $8x10^6$ EL4 cells have reduced tumor growing rate in the group treated with pCIneo-fve plasmid DNA and Fve protein (Square curve). The control group received pCIneo DNA vector alone and 1xPBS (Diamond curve). EL4 tumor formation is observed at day 3. 100μg of pCIneo-fve DNA is intramuscularly injected into the tibialis muscle at days 0 and 7. 20μg of Fve protein is given by subcutaneous injection at days 5, 7, 9, 11, 13, 15, and 18, respectively.

Figure 36. C57BL/6J mice with EL4 solid tumor have extended mean survival time following treatment with the native Fve protein. Eight weeks old female C57BL mice are inoculated with EL4 tumor in the dorsal back. Tumor formation is observed 3 days after inoculation. Red line: 100μg of pCIneo-fve plasmid DNA is intramuscularly injected at the tribilis muscle at days 0 and 7. Mice are received 20μg of native Fve protein treatment by subcutaneous injection surrounding the tumor site at days 5, 7, 9, 11, 13, 15, and 18, respectively. Blue line: Mice received 100μg of pCIneo vector alone and 1xPBS as control group.

Figure 37. C57BL/6J mice with B16-F1 melanoma have extended mean survival time following treatment with native Fve protein. Mice are inoculated with B16-F1 tumor cells in the dorsal back. Tumor formation is observed at day 3. Red line: 200μg of pCIneo-fve plasmid DNA is intramuscularly injected at the tribilis muscle at days -30 and day -1. 50μg of Fve protein is given by subcutaneous injection surrounding the tumor site at days 4, 7, 9, and 12, respectively. Blue line: Mice received 200μg of pCIneo vector and 1xPBS as control group.

Figure 38. B16-Fve transfectant has longer survival rate as comparing with B16-vec transfectant. Two groups of C56BL/6J female mice are inoculated either with $5x10^4$ of B16-Fve (Red line) or $5x10^4$ of B16-vec (Blue line) transfectants in the right flank. Transfectant melanoma solid tumor is established at days 5-7. The fatal rates of mice are recorded and presented as survival curve.

Figure 39. Combined DNA vaccination and Fve gene-transduced melanoma cells synergizes the extension of life span in solid tumor-established mice. C57BL/6J mice are separated into three groups and each group consisted of ten mice. Mice are inoculated with $5x10^4$ of B16-F1 tumor transfectants in the dorsal back. Tumor formation is observed at day 5-7. 100μg of pCIneo-fve plasmid DNA is intramuscularly injected at the right and left tribilis muscle of C57BL/6J at day -77, day -35 and day -21. Mice are subcutaneously injected with $5x10^4$ of B16-Fve transfectants (Red line) and B16-vec transfectant (Green Line) at day 0, respectively. 100μg of pCIneo plasmid DNA is operated as same experimental procedure and mice are subcutaneously injected with $5x10^4$ of B16-vec transfectants as negative control (Blue line).

Figure 40. Strategy of oral primed with Fve protein and intramuscular boosted with plasmid DNA could extend the survival rate of mice with lung metastasis. Two groups of five C57BL/6J mice are given with 10mg/ml of Fve protein in the drinking water at day -35, -28 and -21, and each water providing is maintained consecutively for one week. Mice are intravenously injected with $2x10^4$ of B16-F1 (wild type) melanoma cells at day 0. One week after, mice are intramuscularly injected with 100μg of pCIneo-fve plasmid DNA into

the right and left tribilis muscle, respectively. The mixture of $5x10^4$ of B16-Fve cells lysate plus 10μg of Fve protein (Red line) or 10μg of Fve protein alone (Green line) are subcutaneously injected to mice at the following three weeks. Negative control group of mice received same amount of 1xPBS in the drinking water, intravenously injected with $2x10^4$ of B16-F1 melanoma cells, followed by intramuscularly injected with plasmid DNA vector pCIneo, and finally subcutaneously injected with B16-vec cells lysate plus 1xPBS (Blue line).

Figure 41. Two representative crystals of Fve. Tetragonal crystal is grown in 2% PEG 400, 2.0 M Ammonium Sulfate; 0.1 M Tris-HCl pH 8.5. The crystal dimensions are approximately 1 mm × 0.9 mm × 0.5 mm.

Figure 42. 1° oscillation image of Fve crystal. The edge of the image corresponds to a resolution of 1.4Å. Image displayed with Mosflm/Scala.

Figure 43, 44A, 44B, 44C, 45A and 45B show structures of Fve.

SEQUENCES

Appendix A shows the nucleic acid and/or aminio acid sequences of the deletiion mutants Fve D6-18, Fve D19-33, Fve D34-46, Fve D47-60, Fve D61-72, Fve D73-84, Fve D85-97, Fve D98-106, Fve D107-115, Fve D61-97, Fve p55-100.

Appendix A also shows the nucleic acid and/or aminio acid sequences of the subsitution mutants Fve R27A, Fve G28A, Fve T29A, as well as the fusion proteins Blo t 5-Fve (two-in-one chimeric wild type), Blo t 5-Fve R27A (two-in-one chimeric mutant), Blo t 5-Fve T29A (two-in-one chimeric mutant), Der p 2-Fve R27A (two-in-one chimeric mutant), Der p 2-Fve T29A(two-in-one chimeric mutant), Blo t 5-Der p 2-Fve R27A(three-in-one chimeric mutant).

Appendix A also shows the nucleic acid and/or aminio acid sequences of the Fusion Proteins of Viral Antigen and Fve, HPV E7-FveT29A and HCV Core23-FveT29A, as well as the nucleic acid and/or aminio acid sequences of the Fusion Proteins of Tumor-Associated Antigen and Fve, MAGE3-FveT29A, MART1-FveT29A and CEA-FveT29A.

Appendix A also shows the sequences of the primers Fd6-18F (36 mer), Fd6-18R (36 mer), Fd19-33F(36 mer), Fd19-33R(36 mer), Fd34-46F(36 mer), Fd34-46R(36 mer), Fd47-60F(36 mer), Fd47-60R(36 mer), Fd61-72F(36 mer), Fd61-72R(36 mer), Fd73-84F(36 mer), Fd73-84R(36 mer), Fd85-97F(36 mer), Fd85-97R(36 mer), Fd98-106F (36 mer), Fd98-106R (36 mer), Fd107-115R(39 mer), d(61-97)-F(36mer), d(61-97)-R(36mer), [Fv55-100]-F(48mer), [Fv55-100]-R(42mer), F(R27A)-F (27 mer), F(R27A)-R (27 mer), F(G28A)-F (27 mer), F(G28A)-R (27 mer), F(T29A)-F (27 mer), F(T29A)-R (27 mer), Bt5Fv-F (36mer), Bt5Fv-R (36mer), Dp2Fv-F (36mer), Dp2Fv-R (36mer), Bt5Dp2-F(36mer), Bt5Dp2-R(36mer).

Appendix B shows the sequences of fragments of Fve, which comprise all or part of the RGT motif.

Appendix C shows the crystal coordinates of Fve protein.

The methods and compositions described here may suitably employ any one or more of the sequences shown in the Appendices.

DETAILED DESCRIPTION

We have identified an immunoregulatory protein, designated as native Fve, from *Flammulina velutipes*. The cDNA encoding Fve protein has been isolated and biologically active recombinant Fve has been successfully produced in *E.coli*.

Our studies show that native Fve is capable of inducing high levels of expression of IFN-γ, TNF-α and ICAM-I gene expression in activated human T -and NK cells. It also

up-regulates transcription factors IRF-I and NF-κB (c-Rel), but down-regulates Il.-4. In allergic murine model, mice treated with Der p 2, a major house dust mite allergen from *Dermatophagoides pteronyssinus*, plus native Fve show a significant boost of Der p 2-specific IgG2a production. Native Fve also reduces wheel and erythematic flare formation on Der p 2 skin prick test-positive human subject. We also find that fragments, homologues, variants derivatives of native Fve disclosed here (termed "Fve polypeptides") as well as nucleic acids encoding these, also have such activities.

Furthermore, we show in the Examples that Fve polypeptide and native Fve polypeptide is a potent adjuvant that can be codelivered with specific allergens for desensitization of allergic disorders such as asthma, rhinitis and atopic dermatitis. In addition, Fve selectively induces polarization of NK (natural killer) cells and cytotoxic $CD8^+$ T cells *in vitro* and *in vivo*. We therefore disclose anti-cancer therapies and methods which employ these immunostimulatory or immunomodulatory effects. We disclose *in vivo* animal studies which show that this protein prolongs survival rate in solid tumor-transplanted mice.

Fve and its polypeptides may therefore be used for any application where up-regulation of a immune response is desired or necessary. Fve polypeptides may in particular be used in therapy, for example for the treatment of diseases such as infections, cancer, etc.

We further disclose a combination of Fve polypeptide or native Fve, with an allergen, for example in the form of a fusion protein. Such a combination is able to counteract an established allergic reaction. Combinations of Fve polypeptide or native Fve with a tumour associated protein or viral oncogenic protein may be used to prevent or treat cancer, or specifically for preventing tumour progression.

We disclose immunotherapeutic methods and reagents for allergy and virus infections, which take advantage of these immunomodulatory effects of native Fve and Fve polypeptides. We also disclose methods of treatment or prevention of a cancer,

tumour, neoplasm or cancerous cell, by use of the Fve polypeptides and nucleic acids described here. DNA vaccines, expression vectors, host cells and transgenic organisms comprising such Fve nucleic acids, or a fragment, homologue, variant or derivative thereof, may also be used for such a purpose. In general, any use of a Fve polypeptide described here may employ a nucleic acid encoding such, or a DNA vaccine, expression vector, host cell and transgenic organism comprising such, and the disclosure should be read accordingly.

We also show that native Fve stimulates gene expression of human IFN-γ, TNF-α, IL-1β, IL-2, IRF-1, c-Rel, Bcl-X_L, ICAM-1 and iNOS. In addition, we show that Fve up-regulates a novel subset of $CD8^+$ T cells ($CD3^+$ $CD8^+$ $CD18^{+\,bright}$), and induces NK cell and $CD8^+$ T cell proliferation *in vivo*. Animal studies show that Fve prolongs survival rate of tumor-inoculated mice treated with Fve gene and protein. We disclose methods and reagents for cancer therapy using the Fve gene, protein and products, for example in the form of cell-based vaccines for cancers.

Fve may be used *in vitro* to stimulate the proliferation of $CD3^+$ $CD8^{+bright}$ $CD18^{+bright}$ populations, and the amplified populations may then be administered to the individual in need of treatment. Thus, while it is possible to stimulate $CD3^+$ $CD8^{+bright}$ $CD18^{+bright}$ populations in the context of the body of the animal, it will be apparent that such amplification is also possible *in vitro*. We therefore disclose the use of Fve polypeptides to stimulate such cells *in vitro*. Such amplified populations may then be infused into or otherwise administered to the individual in need of treatment. The starting cell population may come from another individual, but preferably it is derived from the same individual who requires treatment.

We also disclose a crystal of FIP, which has been crystallised for the first time. Such a crystal may be used for modelling, or designing ligands which may interact with Fve. The crystal or model may be stored on a computer, or on a computer readable medium, and manipulated using methods known to the skilled person. A computer readable medium comprising a data representation of the crystal is therefore provided.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; and, D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA* Methods in Enzymology, Academic Press. Each of these general texts is herein incorporated by reference.

NATIVE FVE

The terms "native Fve polypeptide" or "native Fve protein", as used in this document, should be taken to refer to the immunoregulatory protein Fve from *Flammulina velutipes*, preferably in isolated form. The term "wild type Fve" should be understood to be synonymous with "native" Fve; furthermore, the term "nFve" is sometimes used to refer to native Fve.

Preferably, "native" Fve has an amino acid sequence set out as as GenBank accession numbers: S69147 immunomodulatory protein FIP-fve - golden needle mushroom gi|7438667|pir||S69147[7438667] and P80412 IMMUNOMODULATORY PROTEIN FIP-FVE gi|729544|sp|P80412|FVE_FLAVE[729544]. A polypeptide and nucleic acid sequence of "native" or "wild type" Fve is also shown in **Appendix A**, and the term "native FIP" preferably refers to a polypeptide or nucleic acid, as the case may be, having such sequence. Methods of isolating the "native" Fve gene and protein from *Flammulina velutipes* are known in the art, and are also set out in the Examples.

A "native" Fve may comprise a methionine residue at the N terminus; however, a native Fve may include versions which lack the initial methionine. The nucleic acid sequence which encodes such a native Fve may therefore comprise or not comprise an initial ATG codon.

As noted above, we have identified certain previously unknown properties of native Fve, including immunomodulatory and stimulatory properties, and one aspect of the invention is directed to such new uses of native Fve nucleic acid and native Fve polypeptide. These are disclosed in further detail below.

It should be understood, therefore, that the invention preferably does not include wild-type or native Fve protein; however, it does encompass the uses of this in immunomodulation, enhancing immune response and in allergy and cancer treatment. Furthermore, we disclose a fusion protein comprising gluthathione S transferase (GST) and native Fve; such a fusion protein is shown in the Examples to have the beneficial properties of native Fve itself. The sequence of GST-Fve is shown in **Appendix A.** Therefore, the invention includes this GST-Fve fusion protein (also referred to as rGST-Fve and GST-Fve (wild type)), and nucleic acids encoding it.

We further disclose a nucleic acid sequence encoding native Fve, termed here a "native Fve nucleic acid sequence". The Examples describe the cloning and isolation of a cDNA encoding native Fve protein. The sequence of this is set out as "Fve (Wild type)" in **Appendix A.** Preferably such a sequence is in isolated form.

FVE POLYPEPTIDES

Additionally, we have identified various fragments, homologues, variants and derivatives of "native Fve", which are previously unknown. Such fragments, homologues, variants and derivatives are referred to here as "Fve polypeptides" (as contrasted with "native Fve polypeptides"). We disclose such Fve polypeptides, and their uses.

It will be apparent that the terms "Fve" and "Fve polypeptide", as they is used in this document, preferably exclude the wild type or native Fve protein or gene encoding this, and includes only molecules derived from native Fve, being fragments, homologues, variants and derivatives of native Fve (i.e., Fve polypeptides).

The Fve polypeptides are preferably are at least as biologically active as native Fve. However, they may have 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more of the biological activity of native Fve, for example as assayed by any of the tests set out below. As used herein "biologically active" refers to a sequence having a similar structural function (but not necessarily to the same degree), and/or similar regulatory function (but not necessarily to the same degree), and/or similar biochemical function (but not necessarily to the same degree) of the naturally occurring sequence.

"Fve polypeptides" preferably comprise at least one biological activity of native Fve. By "biological activity" in relation to Fve, we refer to at least one of the following activities: up-regulation of expression of Th1 cytokines, preferably IFN-γ and TNF-α, down-regulation of expression of Th2 cytokines, preferably IL-4 and IL-13, hemagglutination activity, cell aggregation activity, lymphocyte aggregation activity, lymphoproliferation activity, up-regulation of expression of IL-2, IFN-γ, TNF-α, but not IL-4 in $CD3^+$ T cells, interaction with T and NK cells, adjuvant activity, stimulation of $CD3^+$ $CD16^+$ $CD56^+$ natural killer (NK) T cells, and up-regulation of expression of allergen specific IgG2a antibody. Further biological activities preferably comprised by Fve polypeptides as described here include prevention of systemic anaphylactic reactions and/or decreased footpad edema, preferably as assayed using the Arthus reaction (Ko et al, 1995). In particular, Fve polypeptides preferably comprise at least some of useful properties, preferably medically or therapeutically useful properties, of native Fve.

Assays for each of these activities are set out in the Examples, and preferably, whether a Fve polypeptide comprises a "biological activity" of Fve is to be assessed according to the relevant assay set out in the Examples.

Preferably, Fve polypeptides comprise at least one or more of the biological activities for the relevant use, preferably use as an immunomodulator, or for upregulating immune response. Preferably, they comprise at least one or more of the biological activities which enable use as a cancer therapy or allergy therapy.

Preferably, Fve polypeptides comprise two or more biological activities of native Fve, preferably substantially all the biological activities of native Fve.

We show in the Examples that the sequence RGT at positions 27-29 of the native Fve polypeptide sequence plays a crucial role in the biological activity of native Fve. In particular, the RGT is shown to mediate the ability of native Fve to cause lymphocyte aggregation and adhesion. This sequence is also shown to mediate lymphoproliferation, and stimulation of IL-2, IFN-γ and TNF-γ secretion in T cells, preferably $CD3^+$ T cells.

Accordingly, in preferred embodiments, the Fve polypeptides comprise at least one, two or all three of the RGT residues (or a functional variant such as RGD) at or about a position corresponding to position 28 of the native Fve polypeptide. By functional variant of RGT, we mean any change in the residues of RGT (or a sequence surrounding it) which does not substantially abolish its function, preferably its function in mediating the activities set out above. Preferably, the Fve polypeptide comprises between 2 to 50, more preferably between 2 to 40, more preferably between 2 to 30, most preferably between 2 to 20 residues of amino acid sequence flanking the glycine residue corresponding to position 28 of native Fve. More preferably, the Fve polypeptide comprises the sequence RGT or the sequence RGD.

However, we show that mutations of R at position 27, as well as mutations of T at position 29, have advantageous effects, in that they independently increase activity of a Fve polypeptide comprising either or both of these mutations. Furthermore, each of the mutations, or in combination, have the potential to increase the solubility of the Fve polypeptide comprising it or them. One, each or both of R27 and T29 may therefore be independently mutated advantageously, by substitution or deletion.

In preferred embodiments, the or each of R27 and T29 are mutated by substitution. The R27 and / or T29 may be substituted by any other residue, but preferably a neutral residue such as G or A. We therefore disclose Fve polypeptides in which R at position 27 is changed to another residue, for example, Fve polypeptides in which R27 is mutated to A, i.e., a Fve polypeptide comprising R27A. We therefore disclose Fve polypeptides in which T at position 29 is changed to another residue, for example, Fve polypeptides in which T29 is mutated to A, i.e., a Fve polypeptide comprising T29A.

Combinations are also possible; hence we disclose Fve polypeptides in which R at position 27 and T at position 29 are independently changed to one or more other residues. For example, we disclose Fve polypeptides in which R27 is mutated to A, and T29 is mutated to A, i.e., a Fve polypeptide comprising R27A and T29A. As noted above, the polypeptide may comprise between 2 to 50, 40, 30 or preferably 20 residues of amino acid flanking the glycine residue at position 28 of native Fve.

Fve polypeptides may comprise fragments of native Fve. For example, Fve D6-18, Fve D19-33, Fve D34-46, Fve D47-60, Fve D61-72, Fve D73-84, Fve D85-97, Fve D98-106, Fve D107-115, Fve D61-97, and Fvep55-100. Fusion proteins comprising these deletion fragments and GST are also disclosed. Fve polypeptides may comprise substitutions, including FveR27A, FveG28A and FveT29A. Further examples of Fve polypeptides are shown in **Appendix B**, each of which includes at least a portion of the RGT sequence (preferably the whole of the RGT sequence) discussed above. Preferably, the length of such a fragment is 9 amino acid residues or more, e.g., fragment numbers 34-403.

Fve polypeptides may comprise fusion proteins, particularly fusion proteins between an allergen and a Fve polypeptide as defined here. Such allergen-immunomodulator combinations include Blo t 5-Fve(two-in-one chimeric wild type), Blo t 5-FveR27A (two-in-one chimeric mutant), Blo t 5-FveT29A (two-in-one chimeric mutant), Der p 2-FveR27A (two-in-one chimeric mutant), Der p 2-FveT29A (two-in-one chimeric mutant) and Blo t 5-Der p 2-FveR27A (three-in-one chimeric mutant).

Fragments, homologues, variants and derivatives of each of these Fve polypeptides are also included.

The Fve polypeptides may be made by biochemical methods, for example, protein digestion of native Fve, or preferably by recombinant DNA methods as known in the art. Accordingly, it will be understood that Fve polypeptides specifically include recombinant Fve polypeptides. For example, we disclose in the Examples successful production in *E.coli* of biologically active recombinant Fve polypeptide.

The Fve polypeptides disclosed also include homologous sequences obtained from any source, for example related viral/bacterial proteins, cellular homologues and synthetic peptides, as well as variants or derivatives thereof. Thus polypeptides also include those encoding homologues of Fve from other species including other microorganisms. Furthermore, homologues from higher animals such as mammals (e.g. mice, rats or rabbits), especially primates, more especially humans are also included.

Homologues

In the context of this document, a "homologous" sequence is taken to include an amino acid sequence which is at least 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% identical, preferably at least 95 or 98% identical at the amino acid level over at least 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110 or 114 amino acids with the sequence of native Fve shown as "Fve (Wild type)" in **Appendix A**. In particular, homology should typically be considered with respect to those regions of the sequence known to be essential for protein function rather than non-essential neighbouring sequences. This is especially important when considering homologous sequences from distantly related organisms.

Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present document it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These publicly and commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues (for example less than 50 contiguous amino acids).

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A; Devereux *et al.*, 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 *ibid* – Chapter 18), FASTA (Atschul *et al.*, 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999 *ibid*, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Advantageously, the BLAST algorithm is employed, with parameters set to default values. The BLAST algorithm is described in detail at http://www.ncbi.nih.gov/BLAST/blast_help.html, which is incorporated herein by reference. The search parameters are defined as follows, can be advantageously set to the defined default parameters.

Advantageously, "substantial identity" when assessed by BLAST equates to sequences which match with an EXPECT value of at least about 7, preferably at least about 9 and most preferably 10 or more. The default threshold for EXPECT in BLAST searching is usually 10.

BLAST (Basic Local Alignment Search Tool) is the heuristic search algorithm employed by the programs blastp, blastn, blastx, tblastn, and tblastx; these programs ascribe significance to their findings using the statistical methods of Karlin and Altschul (Karlin and Altschul 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-68; Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-7; see http://www.ncbi.nih.gov/BLAST/blast_help.html) with a few enhancements. The BLAST programs are tailored for sequence similarity searching, for example to identify homologues to a query sequence. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al* (1994) Nature Genetics 6:119-129.

The five BLAST programs available at http://www.ncbi.nlm.nih.gov perform the following tasks: **blastp** - compares an amino acid query sequence against a protein sequence database; **blastn** - compares a nucleotide query sequence against a nucleotide sequence database; **blastx** - compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database; **tblastn** - compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands); **tblastx** - compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

BLAST uses the following search parameters:

HISTOGRAM - Display a histogram of scores for each search; default is yes. (See parameter H in the BLAST Manual).

DESCRIPTIONS - Restricts the number of short descriptions of matching sequences reported to the number specified; default limit is 100 descriptions. (See parameter V in the manual page).

EXPECT - The statistical significance threshold for reporting matches against database sequences; the default value is 10, such that 10 matches are expected to be found

merely by chance, according to the stochastic model of Karlin and Altschul (1990). If the statistical significance ascribed to a match is greater than the EXPECT threshold, the match will not be reported. Lower EXPECT thresholds are more stringent, leading to fewer chance matches being reported. Fractional values are acceptable. (See parameter E in the BLAST Manual).

CUTOFF - Cutoff score for reporting high-scoring segment pairs. The default value is calculated from the EXPECT value (see above). HSPs are reported for a database sequence only if the statistical significance ascribed to them is at least as high as would be ascribed to a lone HSP having a score equal to the CUTOFF value. Higher CUTOFF values are more stringent, leading to fewer chance matches being reported. (See parameter S in the BLAST Manual). Typically, significance thresholds can be more intuitively managed using EXPECT.

ALIGNMENTS - Restricts database sequences to the number specified for which high-scoring segment pairs (HSPs) are reported; the default limit is 50. If more database sequences than this happen to satisfy the statistical significance threshold for reporting (see EXPECT and CUTOFF below), only the matches ascribed the greatest statistical significance are reported. (See parameter B in the BLAST Manual).

MATRIX - Specify an alternate scoring matrix for BLASTP, BLASTX, TBLASTN and TBLASTX. The default matrix is BLOSUM62 (Henikoff & Henikoff, 1992). The valid alternative choices include: PAM40, PAM120, PAM250 and IDENTITY. No alternate scoring matrices are available for BLASTN; specifying the MATRIX directive in BLASTN requests returns an error response.

STRAND - Restrict a TBLASTN search to just the top or bottom strand of the database sequences; or restrict a BLASTN, BLASTX or TBLASTX search to just reading frames on the top or bottom strand of the query sequence.

FILTER - Mask off segments of the query sequence that have low compositional complexity, as determined by the SEG program of Wootton & Federhen (1993) Computers and Chemistry 17:149-163, or segments consisting of short-periodicity internal repeats, as determined by the XNU program of Claverie & States (1993) Computers and Chemistry 17:191-201, or, for BLASTN, by the DUST program of Tatusov and Lipman (see http://www.ncbi.nlm.nih.gov). Filtering can eliminate statistically significant but biologically uninteresting reports from the blast output (e.g., hits against common acidic-, basic- or proline-rich regions), leaving the more biologically interesting regions of the query sequence available for specific matching against database sequences.

Low complexity sequence found by a filter program is substituted using the letter "N" in nucleotide sequence (e.g., "NNNNNNNNNNNNN") and the letter "X" in protein sequences (e.g., "XXXXXXXXXX").

Filtering is only applied to the query sequence (or its translation products), not to database sequences. Default filtering is DUST for BLASTN, SEG for other programs.

It is not unusual for nothing at all to be masked by SEG, XNU, or both, when applied to sequences in SWISS-PROT, so filtering should not be expected to always yield an effect. Furthermore, in some cases, sequences are masked in their entirety, indicating that the statistical significance of any matches reported against the unfiltered query sequence should be suspect.

NCBI-gi - Causes NCBI gi identifiers to be shown in the output, in addition to the accession and/or locus name.

Most preferably, sequence comparisons are conducted using the simple BLAST search algorithm provided at http://www.ncbi.nlm.nih.gov/BLAST. In some embodiments, no gap penalties are used when determining sequence identity.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

Variants and Derivatives

The terms "variant" or "derivative" in relation to the amino acid sequences disclosed here includes any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acids from or to the sequence providing the resultant amino acid sequence retains substantially the same activity as the unmodified sequence. Preferably, the modified sequence has at least one biological activity as the unmodified sequence, preferably all the biological activities of the unmodified sequence. Preferably, the "variant" or "derivative" has at least one biological activity of native Fve, as described above.

Polypeptides having the amino acid sequence shown in the description and Examples, or fragments or homologues thereof may be modified for use in the methods and compositions described here. Typically, modifications are made that maintain the biological activity of the sequence. Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions provided that the modified sequence retains the biological activity of the unmodified sequence. Alternatively, modifications may be made to deliberately inactivate one or more functional domains of the polypeptides described here. Functional domains of native Fve include the α helix at the N terminus, any of the six β helices, as well as the "loop-like" structures at the N and C termini. Preferably, the functional domain of native Fve comprises the N-terminus helix and the loop/strand, which are essential for protein dimerization.

Amino acid substitutions may include the use of non-naturally occurring analogues, for example to increase blood plasma half-life of a therapeutically administered polypeptide.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

Polypeptides also include fragments of the full length sequence of native Fve, or any of the Fve polypeptides disclosed here. Preferably fragments comprise at least one epitope. Methods of identifying epitopes are well known in the art. Fragments will typically comprise at least 6 amino acids, more preferably at least 10, 20, 30, 50 or 100 amino acids.

Fve polypeptides, fragments, homologues, variants and derivatives, are typically made by recombinant means, for example as described below in the Examples. However they may also be made by synthetic means using techniques well known to skilled persons such as solid phase synthesis. The proteins may also be produced as fusion proteins, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and β-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the function of the protein of interest sequence. Proteins may also be obtained by purification of cell extracts from animal cells.

The Fve polypeptides, variants, homologues, fragments and derivatives disclosed here may be in a substantially isolated form. It will be understood that such polypeptides

may be mixed with carriers or diluents which will not interfere with the intended purpose of the protein and still be regarded as substantially isolated. A Fve variant, homologue, fragment or derivative may also be in a substantially purified form, in which case it will generally comprise the protein in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the protein in the preparation is a protein.

The Fve polypeptides, variants, homologues, fragments and derivatives disclosed here may be labelled with a revealing label. The revealing label may be any suitable label which allows the polypeptide, etc to be detected. Suitable labels include radioisotopes, e.g. ^{125}I, enzymes, antibodies, polynucleotides and linkers such as biotin. Labelled polypeptides may be used in diagnostic procedures such as immunoassays to determine the amount of a polypeptide in a sample. Polypeptides or labelled polypeptides may also be used in serological or cell-mediated immune assays for the detection of immune reactivity to said polypeptides in animals and humans using standard protocols.

A Fve polypeptide, variant, homologue, fragment or derivative disclosed here, optionally labelled, my also be fixed to a solid phase, for example the surface of an immunoassay well or dipstick. Such labelled and/or immobilised polypeptides may be packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like. Such polypeptides and kits may be used in methods of detection of antibodies to the polypeptides or their allelic or species variants by immunoassay.

Immunoassay methods are well known in the art and will generally comprise: (a) providing a polypeptide comprising an epitope bindable by an antibody against said protein; (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

The Fve polypeptides, variants, homologues, fragments and derivatives disclosed here may be used in *in vitro* or *in vivo* cell culture systems to study the role of their corresponding genes and homologues thereof in cell function, including their function in

disease. For example, truncated or modified polypeptides may be introduced into a cell to disrupt the normal functions which occur in the cell. The polypeptides may be introduced into the cell by *in situ* expression of the polypeptide from a recombinant expression vector (see below). The expression vector optionally carries an inducible promoter to control the expression of the polypeptide.

The use of appropriate host cells, such as insect cells or mammalian cells, is expected to provide for such post-translational modifications (e.g. myristolation, glycosylation, truncation, lapidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products. Such cell culture systems in which the Fve polypeptides, variants, homologues, fragments and derivatives disclosed here are expressed may be used in assay systems to identify candidate substances which interfere with or enhance the functions of the polypeptides in the cell.

IMMUNOMODULATOR-ANTIGEN COMBINATIONS AND CONJUGATES

We show throughout this document (for the first time) that Fve has immunomodulatory properties, and in particular can act to potentiate an immune response. The adjuvant property of Fve may be exploited by administering Fve polypeptide or nucleic acid (or a fragment, homologue, variant or derivative thereof, or a host cell or vector comprising such) as described below, along with a molecule to which an immune response is desired.

The Fve polypeptide, etc may be administered to an individual either in combination, sequentially or simultaneously or in succession with the molecule to which an immune response is desired. We therefore provide for the first time a combination of a Fve polypeptide, etc with an antigenic molecule.

Where the Fve polypeptide, etc and the molecule are administered in combination, this may be achieved by administering a mixture of the Fve polypeptide, etc and the

molecule. We therefore provide a simple combination of the Fve polypeptide, etc and the molecule, preferably as a kit. The kit may comprise the Fve polypeptide, etc and the molecule to which an immune response is desired in separate containers, and may optionally comprise instructions to administer these simultaneously, sequentially, etc.

The molecule to which an immune response is desired may comprise an allergen. These are set out in further detail in the following section.

The molecule to which an immune response is desired may comprise a tumour associated antigen. In preferred embodiments, the tumour associated antigen comprises MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, or P15. Nucleic acid and amino acid sequences of these antigens are known in the art, and the skilled person will know how to produce tumour associated antigens, including those set out above. We therefore disclose combinations, preferably in the form of kits, comprising an Fve polypeptide or nucleic acid (or a fragment, homologue, variant or derivative thereof, or a host cell or vector comprising such), together with a tumour associated antigen, for example as set out above.

The molecule to which an immune response is desired may comprise a viral antigen. In preferred embodiments, the viral antigen comprises a protein from an oncogenic virus; such viruses are known in the art. Preferably the oncogenic viral antigen comprises E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; or Tax from HTLV-1.

In a further embodiment, the viral antigen comprises an antigen, preferably a protein, more preferably an antigenic protein or fragment thereof from an infectious virus. Such immunomodulator-viral antigen conjugates may be used to treat or prevent a viral infectious disease, i.e., the cognate disease. For example, an immunomodulator-HSV antigen conjugate, for example, a Fve polypeptide-HSV antigen conjugate, may be used to

treat or prevent Herpes Simplex Virus infection. Other preferred viral antigens include those from Adenovirus, Parainfluenza 3 virus, Human Immunodeficiency Virus (HIV), Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, and Influenza A, Flu A. These viruses, and the diseases they cause, are well known in the art, and methods for making and purifying antigens from such viruses are also well known. For example, US Patent Number 4,313,927 (Fridlender) discloses detailed protocols for preparation of rubella and Cytomegalovirus (CMV) antigen.

Nucleic acid and amino acid sequences of these viral antigens are known in the art, and the skilled person will know how to produce viral antigen antigens, including these set out above. We therefore disclose combinations, preferably in the form of kits, comprising an Fve polypeptide or nucleic acid (or a fragment, homologue, variant or derivative thereof, or a host cell or vector comprising such), together with a viral antigen, for example as set out above.

In preferred embodiments, we provide administration of the Fve polypeptide, etc and the molecule to which an immune response is desired, in which there is some degree of association between the Fve polypeptide, etc and the molecule in question.

We therefore disclose for the first time an an agent which comprises an immunomodulator coupled, fused, mixed, combined, or otherwise joined to an allergen. Such a construct is referred to as a "immunomodulator-allergen conjugate" in this document. In particular, we disclose the use of Fve adjuvanted allergen vaccines, as explained in further detail in Examples 13 and 14.

The coupling, etc between the immunomodulator and the allergen may be permanent or transient, and may involve covalent or non-covalent interactions (including ionic interactions, hydrophobic forces, Van der Waals interactions, etc). The exact mode of coupling is not important, so long as the immunomodulator-allergen conjugate. Accordingly, where reference is made to "comprising", "conjugation", "coupling", etc,

these references should be taken to include any form of interaction between the immunomodulator and the allergen.

Thus, the immunodulator may be a polypeptide which is provided as a fusion protein with the allergen, for example as shown in Example 13 for Fve/Allergen. An expression vector may be constructed by standard recombinant DNA technology to include a nucleotide sequence capable of expressing a immunodulator, such that a fusion protein is expressed comprising the allergen of interest fused to the immunodulator. The expression vector is transfected or transformed into a suitable host for large scale production of fusion protein, by means known in the art. Purification of the fusion protein may also be carried out by known means. Alternatively, or in addition, and as discussed above, the allergen may be physically associated with the immunomodulator, and attached to it by chemical conjugation. Thus, Example 14 below describes the use of allergen physically conjugated to Fve.

In preferred embodiments, the immunomodulator-allergen conjugate is capable of at least one of the following, preferably two or more, more preferably all: increase the number of human PBMC, to stimulate the proliferation of human lymphocytes, to polarize human $CD8^+$ T cells, and to increase the production of IFN-γ (Th1 response) and IL-10 (Tr response). Preferablly, the immunomodulator-allergen conjugate is capable of inducing both Th1 and Tr immune responses. Preferably, the Th1 response inhibits the development of Th2 cells via IFN-γ, more preferably it is capable of inducing a life-long (or substantially long lasting) protective Th1 memory immune response. Allergen specific Tr cells may in turn dampen the anti-allergic Th1 immune response, ensuring a well-balanced protective but nonpathological Th1 response. Allergen-Fve fusion proteins meet these criteria since they induce cytokine IL-10, and these are therefore preferred.

Where the conjugate comprises Fve, the Fve portion of the conjugate may comprise the whole molecule, or fragments of it. It may for example comprise the native Fve, or any Fve polypeptide as disclosed above. The allergen portion may comprise any allergen, whether proteinaceous or not. Advantageously, proteinaceous allergens are conjugated to

the immunomodulator portion by means of covalent bonds, for example, amide bonds (for example, as a fusion protein).

The allergen may comprise for example the whole or a portion of Blo t 5 or Der p 2 allergen. In highly preferred embodiments, the immunomodulator-allergen conjugate comprises Bt5-Fve, Bt5-FveR27 or GST-Dp2-FveR27. Examples of other allergens suitable for use in the immunomodulator-allergen conjugate described here are provided below.

Furthermore, protein-protein conjugation also provides a convenient and alternative choice to develop allergen vaccine. Any suitable means of conjugation, for example, chemical conjugation may be used to couple the immunomodulator and the allergen. Cross-linkers, for example, heterobifunctional cross linkers are known in the art, and may be used. Furthermore, other conjugation agents, for example, poly-lactic acid (PLA) and polyethylene glycol (PEG) may also be employed.

ALLERGENS

In general, the allergen from which an immunomodulator-allergen conjugate may be constructed may come from any source, for example, a source known to induce allergenic responses in humans. For example, the allergen may comprise a tree pollen allergen, a grass pollen allergen, a weed pollen allergen, a feline antigen, or a fungal allergen. Thus, the allergen may comprise a tree pollen allergen, for example Bet v 1 and Bet v 2 from birch tree. It may comprise a grass pollen allergen, for example, Phl p 1 and Phl p 2 from timothy grass. It may comprise a weed pollen allergen, for example, antigen E from ragweed. It may comprise a major feline antigen, for example, Fel d 1. It may comprise a major fungal allergen, for example, Asp f1, Asp f2, and Asp f3 from *Aspergillus fumigatus*.

In preferred embodiments, the allergen comprises a dust mite allergen, preferably a house dust mite allergen. In particular, the allergen is preferably derived from a mite from

Family Glycyphagidae or Family Pyroglyphidae. Dust mites of Family Glycyphagidae include those in the genera Aeroglyphus, Austroglycyphagus, Blomia, Ctenoglyphus, Glycyphagus, Gohieria, Lepidoglyphus. Dust mites of Family Pyroglyphidae include those in the genera Dermatophagoides, Euroglyphus, Pyroglyphus. In preferred embodiments, the allergen is preferably an allergen from a species in any of these genera.

In highly preferred embodiments, the allergen is a group 1 allergen (Der p 1, Der f 1, Blo t 1, Eur m1, Lep d 1), a group 2 allergen (Der p 2, Der f 2, Blo t 2, Eur m 2, Lep d 2), a group 5 allergen (Blo t 5, Der p 5, Der f 5, Eur m 5, Lep d 5) or a group 15 allergen (Der p 15, Der f 15, Blot 15, Eur m 15, Lep d 15) from dust mite. Nucleic acid and amino acid sequences of these allergens are known in the art, and the skilled person will know how to produce allergen-immunomodulator conjugates from any of these allergens using such sequences.

OTHER IMMUNOMODULATOR CONJUGATES

Immunomodulator-Tumour Associated Antigen Conjugates

We also disclose for the first time an an agent which comprises an immunomodulator coupled, fused, mixed, combined, or otherwise joined to an tumour associated antigen. Such a construct is referred to as a "immunomodulator-tumour associated antigen conjugate" in this document.

As the term is used here, "tumour associated antigen" generally includes a cancer protein or a cancer antigen, i.e., a protein which is preferentially expressed in a tumour cell or a transformed cell, compared to a "normal" non-cancerous cell.

In highly preferred embodiments, the tumour associated antigen may comprise MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, or P15. Nucleic acid and amino acid sequences

of these antigens are known in the art, and the skilled person will know how to produce tumour associated antigen-immunomodulator conjugates from any of these allergens using such sequences.

We present in **Appendix A** the sequences of MAGE3-FveT29A, MART1-FveT29A and CEA-FveT29A, which are preferred Immunomodulator-Tumour Associated Antigen Conjugates suitable for use in the methods and compositions described here.

Immunomodulator-Viral Antigen Conjugates

We further disclose an agent comprising an immunodulator coupled, etc to a viral antigen. In highly preferred embodiments, the viral antigen comprises a protein from an oncogenic virus; such viruses are known in the art. Preferably the oncogenic viral antigen comprises E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; or Tax from HTLV-1. Nucleic acid and amino acid sequences of these viral antigens are known in the art, and the skilled person will know how to produce viral antigen-immunomodulator conjugates from any of these allergens using such sequences.

We also provide an agent (for example a polypeptide) comprising a first portion comprising at least a portion of Fve and a second portion comprising at least a portion of a viral antigen, preferably coupled together. The viral antigen may be selected from the group consisting of antigens from Adenovirus, Parainfluenza 3 virus, Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, and Influenza A, Flu A.

The viral antigen may comprise any portion of the native viral antigen, for example, a portion of the HCV core antigen. We have established that a deletion of the HCV core antigen, particularly a deletion of 23 amino acids from residues 141 to 163 of the core antigen leads to an increase in efficiency of protein production. Accordingly, we provide an agent comprising an immunodulator coupled, etc to a viral antigen, which viral antigen comprises such a deleted core antigen (here referred to as "Core23"), e.g., the fusion protein HCV Core23-FveT29A.

In particular, we find that the polypeptides HCV Core23-FveT29A and HPV E7-FveT29A (the sequences of which are shown in **Appendix A**) are particularly useful as Immunomodulator-Viral Antigen conjugates.

The coupling, etc between the immunomodulator and the tumour associated antigen, and the viral antigen, may be as described above for the immunomodulator-allergen conjugate.

FVE NUCLEIC ACIDS

We provide for a nucleic acid encoding a Fve polypeptide, which we refer to as a "Fve nucleic acid". We also provide nucleic acids encoding variants, homologues, derivatives and fragments of native Fve, as well as fragments, homologues, derivatives and variants of Fve nucleic acids.

Preferably, the Fve nucleic acid is derived from a natural or native Fve sequence, for example, the nucleic sequence shown as "Fve (Wild type)" in **Appendix A**. In a preferred embodiment, the Fve nucleic acid is a recombinant fragment of native Fve nucleic acid, or any fragment, homologue, variant or derivative thereof. Fragments, homologues, variants and derivatives of each of the above sequences are also included.

"Fve nucleic acids" preferably encode polypeptides which have at least one biological activity of native Fve, as described above. Preferably, Fve nucleic acids encode polypeptides which comprise two or more biological activities of native Fve, preferably substantially all the biological activities of native Fve.

In preferred embodiments, the Fve nucleic acids encode polypeptides which comprise at least one, two or all three of the RGT residues (or a functional variant as defined above, such as RGD) at or about a position corresponding to position 28 of the native Fve polypeptide. In particular, the Fve nucleic acid may comprise the sequence CGTGGTACC. Alternatively, the Fve nucleic acid may comprise the sequence

CGTGGTGAT or the sequence CGTGGTGAC. The Fve nucleic acid may comprise a nucleotide sequence which encodes the same amino acids as a result of the redundancy of the genetic code.

The Fve nucleic acid maycomprise a sequence comprising three codons, with a first codon selected from the group consisting of: CGT, CGC, CGA, CGG, AGA and AGG, a second codon selected from the group consisting of: GGT, GGC, GGA and GGG, and a third codon selected from the group consisting of: ACT, ACC, ACA and ACG. Alternatively, the third codon may be selected from the group consisting of: GAT and GAC,

Preferably, the Fve polypeptide comprises between 2 to 60 residues of nucleic acid sequence flanking the codon for the glycine residue corresponding to position 28 of native Fve.

In preferred embodiments, Fve nucleic acids may comprise nucleic acids encoding fragments of native Fve. For example, Fve nucleic acids may comprise the nucleic acid sequences depicted in **Appendix A** as Fve D6-18, Fve D19-33, Fve D34-46, Fve D47-60, Fve D61-72, Fve D73-84, Fve D85-97, Fve D98-106, Fve D107-115, Fve D61-97, and Fvep55-100. Nucleic acids encoding fusion proteins comprising these deletion fragments and GST are also disclosed. Fve nucleic acids may comprise those encoding substitutions, including FveR27A, FveG28A and FveT29A. Fve nucleic acids include those which encode the polypeptide sequences shown in **Appendix A**.

We also disclose Fve nucleic acids which encode Fve polypeptides comprising fusion proteins, particularly fusion proteins between an allergen and a Fve polypeptide as defined here. We disclose in particular nucleic acid sequences of Blo t 5-Fve(two-in-one chimeric wild type), Blo t 5-FveR27A (two-in-one chimeric mutant), Blo t 5-FveT29A (two-in-one chimeric mutant), Der p 2-FveR27A (two-in-one chimeric mutant), Der p 2-FveT29A (two-in-one chimeric mutant) and Blo t 5-Der p 2-FveR27A (three-in-one chimeric mutant), and shown in **Appendix A**.

As used here in this document, the terms "polynucleotide", "nucleotide", and nucleic acid are intended to be synonymous with each other. "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

It will be understood by a skilled person that numerous different polynucleotides and nucleic acids can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described here to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed.

Fve nucleic acids, variants, fragments, derivatives and homologues may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of this document, it is to

be understood that the polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides of interest.

The terms "variant", "homologue" or "derivative" in relation to a nucleotide sequence include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence. Preferably said variant, homologues or derivatives code for a polypeptide having biological activity.

As indicated above, with respect to sequence homology, preferably there is at least 50 or 75%, more preferably at least 85%, more preferably at least 90% homology to the sequences shown in the sequence listing herein. More preferably there is at least 95%, more preferably at least 98%, homology. Nucleotide homology comparisons may be conducted as described above. A preferred sequence comparison program is the GCG Wisconsin Bestfit program described above. The default scoring matrix has a match value of 10 for each identical nucleotide and -9 for each mismatch. The default gap creation penalty is -50 and the default gap extension penalty is -3 for each nucleotide.

We further describe nucleotide sequences that are capable of hybridising selectively to the sequences presented herein, or any variant, fragment or derivative thereof, or to the complement of any of the above. Nucleotide sequences are preferably at least 15 nucleotides in length, more preferably at least 20, 30, 40 or 50 nucleotides in length.

The term "hybridization" as used herein shall include "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" as well as the process of amplification as carried out in polymerase chain reaction technologies.

Polynucleotides capable of selectively hybridising to the nucleotide sequences presented herein, or to their complement, will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% or 98% homologous to the corresponding

nucleotide sequences presented herein over a region of at least 20, preferably at least 25 or 30, for instance at least 40, 60 or 100 or more contiguous nucleotides.

The term "selectively hybridizable" means that the polynucleotide used as a probe is used under conditions where a target polynucleotide is found to hybridize to the probe at a level significantly above background. The background hybridization may occur because of other polynucleotides present, for example, in the cDNA or genomic DNA library being screening. In this event, background implies a level of signal generated by interaction between the probe and a non-specific DNA member of the library which is less than 10 fold, preferably less than 100 fold as intense as the specific interaction observed with the target DNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P.

Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA), and confer a defined "stringency" as explained below.

Maximum stringency typically occurs at about Tm-5°C (5°C below the Tm of the probe); high stringency at about 5°C to 10°C below Tm; intermediate stringency at about 10°C to 20°C below Tm; and low stringency at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

In a preferred aspect, we provide nucleotide sequences that can hybridise to the Fve nucleic acids, fragments, variants, homologues or derivatives dislosed here under stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na_3 Citrate pH 7.0).

Where the polynucleotide is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the methods and compositions described here. Where the polynucleotide is single-stranded, it is to be understood that the complementary sequence of that polynucleotide is also included.

Polynucleotides which are not 100% homologous to the Fve sequences disclosed here but which are also included can be obtained in a number of ways. Other variants of the sequences may be obtained for example by probing DNA libraries made from a range of individuals, for example individuals from different populations. For example, Fve homologues may be identified from other individuals, or other species. Further recombinant Fve nucleic acids and polypeptides may be produced by identifying corresponding positions in the homologues, and synthesising or producing the molecule as described elsewhere in this document. Furthermore, the collagen region, neck region and carbohydrate binding domain in such homologues may be identified, for example, by sequence gazing or computer assisted comparisons, and selected for combination into or production of a recombinant Fve which has one or more biological activities of native Fve.

In addition, other viral/bacterial, or cellular homologues of Fve particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to Fve. Such homologues may be used to design non-human Fve nucleic acids, fragments, variants and homologues. Mutagenesis may be carried out by means known in the art to produce further variety.

Sequences of Fve homologues may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal or non-animal species, particularly microbial or fungal species, and probing such libraries with probes comprising all or part of any of the Fve nucleic acids, fragments, variants and homologues, or other fragments of Fve under conditions of medium to high stringency.

Similar considerations apply to obtaining species homologues and allelic variants of the polypeptide or nucleotide sequences disclosed here.

Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of the Fve nucleic acids. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used.

The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences. It will be appreciated by the skilled person that overall nucleotide homology between sequences from distantly related organisms is likely to be very low and thus in these situations degenerate PCR may be the method of choice rather than screening libraries with labelled fragments the Fve sequences.

In addition, homologous sequences may be identified by searching nucleotide and/or protein databases using search algorithms such as the BLAST suite of programs.

Alternatively, such polynucleotides may be obtained by site directed mutagenesis of characterised sequences, for example, Fve nucleic acids, or variants, homologues, derivatives or fragments thereof. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides.

The polynucleotides described here may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 8, 9, 10, or 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term "polynucleotides" as used herein.

Polynucleotides such as a DNA polynucleotides and probes may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

Longer polynucleotides will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the lipid targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector

Polynucleotides or primers may carry a revealing label. Suitable labels include radioisotopes such as ^{32}P or ^{35}S, enzyme labels, or other protein labels such as biotin. Such labels may be added to polynucleotides or primers and may be detected using by techniques known *per se*. Polynucleotides or primers or fragments thereof labelled or

unlabeled may be used by a person skilled in the art in nucleic acid-based tests for detecting or sequencing polynucleotides in the human or animal body.

Such tests for detecting generally comprise bringing a biological sample containing DNA or RNA into contact with a probe comprising a polynucleotide or primer under hybridising conditions and detecting any duplex formed between the probe and nucleic acid in the sample. Such detection may be achieved using techniques such as PCR or by immobilising the probe on a solid support, removing nucleic acid in the sample which is not hybridised to the probe, and then detecting nucleic acid which has hybridised to the probe. Alternatively, the sample nucleic acid may be immobilised on a solid support, and the amount of probe bound to such a support can be detected. Suitable assay methods of this and other formats can be found in for example WO89/03891 and WO90/13667.

Tests for sequencing nucleotides, for example, the Fve nucleic acids, involve bringing a biological sample containing target DNA or RNA into contact with a probe comprising a polynucleotide or primer under hybridising conditions and determining the sequence by, for example the Sanger dideoxy chain termination method (see Sambrook *et al.*).

Such a method generally comprises elongating, in the presence of suitable reagents, the primer by synthesis of a strand complementary to the target DNA or RNA and selectively terminating the elongation reaction at one or more of an A, C, G or T/U residue; allowing strand elongation and termination reaction to occur; separating out according to size the elongated products to determine the sequence of the nucleotides at which selective termination has occurred. Suitable reagents include a DNA polymerase enzyme, the deoxynucleotides dATP, dCTP, dGTP and dTTP, a buffer and ATP. Dideoxynucleotides are used for selective termination.

PROTEIN EXPRESSION AND PURIFICATION

Host cells comprising polynucleotides may be used to express polypeptides, such as Fve polypeptides, fragments, homologues, variants or derivatives thereof. Host cells may be cultured under suitable conditions which allow expression of the proteins. Expression of the polypeptides may be constitutive such that they are continually produced, or inducible, requiring a stimulus to initiate expression. In the case of inducible expression, protein production can be initiated when required by, for example, addition of an inducer substance to the culture medium, for example dexamethasone or IPTG.

Polypeptides can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical and/or osmotic lysis and physical disruption.

Polypeptides may also be produced recombinantly in an *in vitro* cell-free system, such as the TnT™ (Promega) rabbit reticulocyte system.

FVE NUCLEIC ACID MOLECULES

We disclose a nucleic molecule that: a) has a strand that encodes an Fve polypeptide disclosed here, b) has a strand that is complementary with a strand as described in a) above; or c) has a strand that hybridises with a molecule as described in a) or b) above.

Unless the context indicates otherwise, such nucleic acid molecules, which are included within the term "Fve nucleic acid molecule" may have one or more of the following characteristics:

1) They may be DNA or RNA (including variants of naturally occurring DNA or RNA structures, which have non-naturally occurring bases and/or non-naturally occurring backbones).

2) They may be single-stranded or double-stranded (or in some cases higher stranded, e.g. triple- stranded).

3) They may be provided in recombinant form i.e. covalently linked to a heterologous 5' and/or 3' flanking sequence to provide a chimeric molecule (e.g. a vector) that does not occur in nature.

4) They may be provided with or without 5' and/or 3' flanking sequences that normally occur in nature.

5) They may be provided in substantially pure form, e.g. by using probes to isolate cloned molecules having a desired target sequence or by using chemical synthesis techniques. Thus they may be provided in a form that is substantially free from contaminating proteins and/or from other nucleic acids.

6) They may be provided with introns (e.g. as a full-length gene) or without introns (e.g. as DNA).

7) They may be provided in linear or non-linear (e.g. circular) form.

These Fve molecules include not only molecules with classical DNA or RNA structures, but also variants with modified (non-phosphodiester) backbones - e.g. morpholino derivatives and peptide nucleic acids (PNAs), which contain an N-(2-aminoethyl)glycine-based pseudopeptide backbone. (See Nielsen, P.E., Annual Review of Biophysics & Biomolecular Structure, 24:167-83 (1995)). Nucleic acid variants with modified backbones can have increased stability relative to unmodified nucleic acids and are particularly useful where hybridisation is desired over a relatively long period (e.g. in antisense therapy).

Nucleic acid molecules and uses thereof are discussed in further detail below:

a) Coding nucleic acid molecules

The Fve polypeptides can be coded for by a large variety of nucleic acid molecules, taking into account the well-known degeneracy of the genetic code. All of these coding nucleic acid molecules are within the scope of the present document.

The Fve nucleic acids may be administered to an individual and used to express polypeptides disclosed here. Thus, they may be used for the same treatments as the Fve polypeptides.

The Fve nucleic acid molecules may be provided in the form of vectors, although this is not essential. Preferred vectors for use in treatment include replication-deficient adenoviruses, retroviruses and adeno-associated viruses.

Fve nucleic acid molecules may be administered to a patient by physical methods. These methods include topical application of the nucleic acid in an appropriate vehicle, for example in solution in a pharmaceutically acceptable excipient, such as phosphate buffered saline (PBS). They also include particle bombardment (which is sometimes known as "gene gun" technology and is described in US Patent No. 5371015). Here inert particles, such as gold beads coated with a nucleic acid, can be accelerated at speeds sufficient to enable them to penetrate cells. They can be used for example to penetrate the skin of a patient and may be administered by means of discharge under high pressure from a projecting device. Other physical methods of administering the Fve nucleic acid directly to a recipient include ultrasound, electrical stimulation (including iontophoresis) and microseeding (see e.g. US Patent No. 5697901). Alternatively, the Fve nucleic acid molecules may simply be injected at appropriate site (e.g. muscle). They may be incorporated in or on a carrier (which may be a lipid-based carrier, such as a liposome).

Fve nucleic acid molecules may be introduced into host cells (optionally in the form of vectors) to enable the expression of polypeptides. Alternatively, cell-free expression systems may be used. By using an appropriate expression system the Fve polypeptides can be produced in a desired form. For example, the Fve polypeptides can be

produced by micro-organisms such as bacteria or yeast, by cultured insect cells (which may be baculovirus-infected), by mammalian cells (such as CHO cells) or by transgenic animals that, for instance, secrete the Fve proteins in milk (see e.g. international patent application WO88/00239). Where glycosylation is desired, eukaryotic (e.g. mammalian or insect) expression systems are preferred.

Whatever means is used to obtain expression, transcriptional and translational control sequences will normally be present and will be operatively linked to a sequence encoding a polypeptide to be expressed. These control sequences may be heterologous to the sequence encoding the Fve polypeptide or may be found associated with it *in vivo*. Promoter, operator and /or enhancer sequences may, for example, be provided, as may polyadenylation sites, splice sites, stop and start codons, upstream and downstream regulatory regions, etc. If desired, a constitutive promoter may be provided. Alternatively, a regulatable promoter may be provided to enable transcription to be controlled by administration of a regulator. The promoter (if present) may be tissue-specific or non tissue-specific.

Polypeptides comprising N-terminal methionine may be produced using certain expression systems, whilst in others the mature polypeptide may lack this residue. Fve polypeptides may initially be expressed so as to include signal sequences. Different signal sequences may be provided for different expression systems. Alternatively, signal sequences may be absent, if not needed.

Once expressed, Fve polypeptides may be purified by a wide variety of techniques. Purification techniques may be used under reducing conditions (in order prevent disulphide bond formation) or non-reducing conditions. Available purification techniques include, for example, electrophoretic techniques, such as SDS PAGE (see e.g. Hunkapiller *et al*, *Methods Enzymol.* 91:227 (1983), which discloses "Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid sequence analysis."); affinity techniques (e.g. immunoaffinity chromatography); HPLC; gel filtration; ion-exchange

chromatography; isoelectric focussing; etc. If desired, combinations of different purification steps may be used and/or individual purification steps may be repeated.

In summary, techniques for cloning, expressing and purifying polypeptides are well known to the skilled person. Various such techniques are disclosed in standard text-books, such as in Sambrook *et al* [*Molecular Cloning* 2nd Edition, Cold Spring Harbor Laboratory Press (1989)]; in Old & Primrose [*Principles of Gene Manipulation* 5th Edition, Blackwell Scientific Publications (1994)]; and in Stryer [*Biochemistry* 4th Edition, W H Freeman and Company (1995)].

b) Complementary nucleic acid molecules

We also describe nucleic acid strands complementary thereto, whether or not the coding and complementary strands are associated in a duplex. Thus, for example, mRNA and cDNA molecules are included.

c) Hybridising nucleic acid molecules

Nucleic acid molecules that can hybridise to one or more of the Fve nucleic acid molecules discussed above are also disclosed. Such nucleic acid molecules are referred to herein as "hybridising" nucleic acid molecules. Desirably hybridising molecules are at least 10 nucleotides in length and preferably are at least 20, at least 50, at least 100, or at least 200 nucleotides in length.

A hybridising nucleic acid molecule may have a high degree of sequence identity along its length with a nucleic acid molecule within the scope of b) or a) above (e.g. at least 50%, at least 75% or at least 90% sequence identity), although this is not essential. The greater the degree of sequence identity that a given single stranded nucleic acid molecule has with a strand of a nucleic acid molecule, the greater the likelihood that it will hybridise to the complement of said strand.

Most preferably, hybridising nucleic acid molecules hybridise to either DNA strand of a Fve nucleic acid, for example a sequence shown in **Appendix A**, or to an RNA equivalent thereof, or to a strand that is complementary to either of the aforesaid strands.

Hybridising nucleic acid molecules can be useful as probes or primers, for example.

Probes can be used to purify and/or to identify Fve nucleic acids. They may be used in diagnosis. For example, probes may be used to determine whether or not an organism such as a fungus has a wild-type gene encoding a Fve polypeptide described here, or whether or not one or more deletions, insertions and/or replacements of bases relative to the wild-type sequence are present. It may therefore be used to identify organisms that do not express Fve polypeptides or that express Fve polypeptides having reduced activity (including inactive polypeptides).

Primers are useful in synthesising nucleic acids or parts thereof based upon a template to which a probe hybridises. They can be used in techniques such as PCR to provide large numbers of nucleic acid molecules.

Hybridising molecules also include antisense strands. These hybridise with "sense" strands so as to inhibit transcription and /or translation. An antisense strand can be synthesised based upon knowledge of a sense strand and base pairing rules. It may be exactly complementary with a sense strand, although it should be noted that exact complementarity is not always essential. It may also be produced by genetic engineering, whereby a part of a DNA molecule is provided in an antisense orientation relative to a promoter and is then used to transcribe RNA molecules. Large numbers of antisense molecules can be provided (e.g. by cloning, by transcription, by PCR, by reverse PCR, etc.

Hybridising molecules include ribozymes. Ribozymes can also be used to regulate expression by binding to and cleaving RNA molecules that include particular target sequences recognised by the ribozymes. Ribozymes can be regarded as special types of

antisense molecule. They are discussed, for example, by Haselhoff and Gerlach (Nature (1988) 334:585 – 91).

Antisense molecules may be DNA or RNA molecules. They may be used in antisense therapy to prevent or reduce undesired expression or activity. Antisense molecules may be administered directly to a patient (e.g. by injection). Alternatively, they may be synthesised *in situ* via a vector that has been administered to a patient.

In addition to the uses described above, the Fve nucleic acid molecules disclosed here (of whatever nature) may be used in screening. Screening can be done to identify moieties that bind to said nucleic acid molecules (e.g. to identify hybridising molecules). It can also be done to identify moieties that affect transcription or translation from said nucleic acid molecules.

It can be used to analyse expression, including analysing expression levels or expression patterns (e.g. by analysing mRNA or cDNA), etc. It can be used to identify particular nucleic acid molecules in a sample. This is useful for in identifying biological material from a given source (e.g. from a human or non-human animal). For example, a reference nucleic acid molecule (or part of it) can be digested with restriction enzymes and the resultant nucleic acid fragments can be run on a gel. This can provide a restriction fragment pattern or "fingerprint" that can be compared with a sample. If the comparison provides a match that is unlikely to have occurred by chance, a conclusion can be reached that the sample and the reference molecule are likely to have originated from a common source. By performing statistical analysis a specific degree of confidence that such a conclusion is correct can be provided.

We also describe a library having a Fve nucleic acid molecule described here, as well as an array comprising such an Fve nucleic acid molecule (which may be a library). Preferably the array is a regular array. The array may have a predetermined pattern. It may have a grid-like pattern. The discussion provided herein in respect of libraries and arrays

comprising a polypeptide described here applies *mutatis mutandis* to libraries and arrays comprising the corresponding nucleic acid molecule.

One or more Fve nucleic acid molecules may be immobilised upon a surface (e.g. the surface of a bead or a chip). The surface may, for example, be silicon surface, glass, quartz, a membrane, etc. Techniques for immobilising nucleic acid molecules upon a surface are known and are disclosed, for example, in EP-A-0487104, WO96/04404, WO90/02205, WO96/12014, WO98/44151. In some cases they may include a step of nucleic acid amplification, which may involve PCR. Immobilisation is not however essential. For example nucleic acids may be provided in wells or other containment means (e.g. in a fluid environment).

The Fve nucleic acids may be used in various ways. For example, sequence information can be used in predicting structure and/or function, in homology or identity studies, etc.

VECTORS

As indicated above the nucleic acid molecules described here may be provided in the form of vectors.

Vectors comprising such nucleic acid include plasmids, phasmids, cosmids, viruses (including bacteriophages), YACs, PACs, etc. They will usually include an origin of replication and may include one or more selectable markers e.g. drug resistance markers and/or markers enabling growth on a particular medium. A vector may include a marker that is inactivated when a nucleic acid molecule, such as the ones described here, is inserted into the vector. Here a further marker may be provided that is different from the marker that is inactivated (e.g. it encodes a different type of drug resistance).

Vectors may include one or more regions necessary for transcription of RNA encoding a polypeptide. Such vectors are often referred to as expression vectors. They will

usually contain a promoter and may contain additional regulatory regions – e.g. operator sequences, enhancer sequences, etc. Translation can be provided by a host cell or by a cell free expression system.

Vectors need not be used for expression. They may be provided for maintaining a given nucleic acid sequence, for replicating that sequence, for manipulating, it or for transferring it between different locations (e.g. between different organisms).

Large nucleic acid molecules may be incorporated into high capacity vectors (e.g. cosmids, phasmids, YACs or PACs). Smaller nucleic acid molecules may be incorporated into a wide variety of vectors.

Fve polynucleotides, for example those described here, can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, we provide a method of making polynucleotides by introducing a polynucleotide into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells include bacteria such as *E. coli*, yeast, mammalian cell lines and other eukaryotic cell lines, for example insect Sf9 cells.

Preferably, a polynucleotide in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" means that the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators.

Vectors may be transformed or transfected into a suitable host cell as described below to provide for expression of a protein. This process may comprise culturing a host cell transformed with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the protein, and optionally recovering the expressed protein. Vectors will be chosen that are compatible with the host cell used.

The vectors may be for example, plasmid or virus vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used, for example, to transfect or transform a host cell.

Control sequences operably linked to sequences encoding the polypeptide include promoters/enhancers and other expression regulation signals. These control sequences may be selected to be compatible with the host cell for which the expression vector is designed to be used in. The term promoter is well-known in the art and encompasses nucleic acid regions ranging in size and complexity from minimal promoters to promoters including upstream elements and enhancers.

The promoter is typically selected from promoters which are functional in mammalian cells, although prokaryotic promoters and promoters functional in other eukaryotic cells, such as insect cells, may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression is to occur. With respect to eukaryotic promoters, they may be promoters that function in a ubiquitous manner (such as

promoters of α-actin, β-actin, tubulin) or, alternatively, a tissue-specific manner (such as promoters of the genes for pyruvate kinase). They may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter, the rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter.

It may also be advantageous for the promoters to be inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated.

In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

Polynucleotides may also be inserted into the vectors described above in an antisense orientation to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides may also be produced by synthetic means. Such antisense polynucleotides may be used in a method of controlling the levels of RNAs transcribed from genes comprising any one of the polynucleotides described here.

HOST CELLS

Vectors and polynucleotides or nucleic acids comprising or encoding Fve nucleic acids, fragments, homologues, variants or derivatives thereof may be introduced into host cells for the purpose of replicating the vectors/polynucleotides and/or expressing the polypeptides encoded by the polynucleotides. Although the polypeptides may be produced using prokaryotic cells as host cells, it is preferred to use eukaryotic cells, for example yeast, insect or mammalian cells, in particular mammalian cells.

Vectors/polynucleotides may be introduced into suitable host cells using a variety of techniques known in the art, such as transfection, transformation and electroporation. Where vectors/polynucleotides are to be administered to animals, several techniques are known in the art, for example infection with recombinant viral vectors such as retroviruses, herpes simplex viruses and adenoviruses, direct injection of nucleic acids and biolistic transformation.

We therefore further disclose cells comprising Fve nucleic acid molecules or vectors. These may for example be used for expression, as described herein.

A cell capable of expressing a Fve polypeptide described here can be cultured and used to provide the Fve polypeptide, which can then be purified.

Alternatively, the cell may be used in therapy for the same purposes as the Fve polypeptide. For example, cells may be provided from a patient (e.g. via a biopsy), transfected with a nucleic acid molecule or vector and, if desired, cultured *in vitro*, prior to being returned to the patient (e.g. by injection). The cells can then produce the Fve polypeptide *in vivo*. Preferably the cells comprise a regulatable promoter enabling transcription to be controlled via administration of one or more regulator molecules. If desired, the promoter may be tissue specific.

Expression is not however essential since the cells may be provided simply for maintaining a given nucleic acid sequence, for replicating the sequence, for manipulating it, etc.

Such cells may be provided in any appropriate form. For example, they may be provided in isolated form, in culture, in stored form, etc. Storage may, for example, involve cryopreservation, buffering, sterile conditions, etc. Such cells may be provided by gene cloning techniques, by stem cell technology or by any other means. They may be part of a tissue or an organ, which may itself be provided in any of the forms discussed above. The cell, tissue or organ may be stored and used later for implantation, if desired.

Techniques for providing tissues or organs, include stem cell technology, the provision of cells tissues or organs from transgenic animals, retroviral and non-retroviral techniques for introducing nucleic acids, etc.

In some case cells may be provided together with other material to aid the structure or function or of an implant. For example scaffolds may be provided to hold cells in position, to provide mechanical strength, etc. These may be in the form of matrixes of biodegradable or non-biodegradable material. WO95/01810 describes various materials that can be used for this purpose.

ANIMALS

We also disclose transgenic animals, preferably non-human transgenic animals. Such animals may be useful for producing the particular Fve polypeptides described here (e.g. via secretion in milk, as described herein). Alternatively, they may be useful as test animals for analysing the effect(s) of such Fve polypeptides.

Techniques for producing transgenic animals are well known and are described e.g. in US patents 4870009 and 4873191. For example, a nucleic acid encoding a Fve polypeptide of interest may be microinjected into a pronucleus of a fertilised oocyte. The oocyte may then be allowed to develop in a pseudopregnant female foster animal. The animal resulting from development of the oocyte can be tested (e.g. with antibodies) to determine whether or not it expresses the particular polypeptide. Alternatively, it can be tested with a probe to determine if it has a transgene (even if there is no expression).

A transgenic animal can be used as a founder animal, which may be bred from in order to produce further transgenic animals. Two transgenic animals may be crossed. For example, in some cases transgenic animals may be haploid for a given gene and it may be desired to try to provide a diploid offspring via crossing.

A transgenic animal may be cloned, e.g. by using the procedures set out in WO97/07668 and WO97/07699 (see also Nature 385:810-813 (1997)). Thus a quiescent cell can be provided and combined with an oocyte from which the nucleus has been removed combined. This can be achieved using electrical discharges. The resultant cell can be allowed to develop in culture and can then be transferred to a pseudopregnant female.

ANALYTICAL TOOLS AND SYSTEMS

We disclose a moiety comprising a Fve polypeptide, a Fve nucleic acid, a vector comprising Fve, a cell expressing Fve, an Fve binding agent, a moiety identified/identifiable by a screen as described here, when used as an analytical tool or when present in a system suitable for analysis, especially high throughput analysis.

Such an analytical tool or system is useful for a plethora of different purposes. These include diagnosis, forensic science, screening, the identification or characterisation of individuals or populations, preventative medicine, etc.

Libraries comprising such a Fve moiety may be used for the above purposes. A library will generally comprise a plurality of heterologous moieties. Preferred libraries comprise at least 100, at least 10,000, at least 1,000,000, or at least 1,000,000,000 heterologous moieties. Desirably a moiety is provided at a predetermined position within a library. In some cases a plurality of moieties may be present within a library at predetermined positions. A predetermined position may be assigned spatial co-ordinates. These may be stored or processed in a computer in order to assist in analysis.

We further disclose an array comprising such a Fve moiety (whether or not the array is also a library). Preferably the array is a regular array. The array may have a predetermined pattern. It may have a grid-like pattern. Preferred arrays comprise at least 100, at least 10,000, at least 1,000,000, or at least 1,000,000,000 components.

A library or array may include naturally occurring moieties, non-naturally occurring moieties, or a mixture of naturally occurring and non-naturally occurring moieties. The moieties may provided in solution, on beads, on chips (see e.g. Fodor (1993) Nature 364:555-556), on bacteria (see e.g. US Patent 5223409), on spores (see e.g. US Patent 5223409), on 'phage (see e.g. Scott and Smith (1990) Science 249:386-90 and US Patent 5223409), etc.

Such Fve moieties may be immobilised upon a surface, if desired. For example, one or more nucleic acid molecules may be immobilised upon a surface (e.g. the surface of a bead or a chip). The surface may, for example, be silicon, glass, quartz, a membrane, etc. Techniques for immobilising nucleic acid molecules upon a surface are known and are disclosed, for example, in EP-A-0487104, WO96/04404, WO90/02205, WO96/12014, WO98/44151. In some cases they may include a step of nucleic acid amplification, and may involve PCR.

Immobilisation is not however essential, even if moieties are to be used in high throughput analysis. For example, they may be provided in wells, channels, grooves or other containment means.

Whether or not present in a library, an array or in immobilised or non-immobilised form, it is often desirable to locate the position of one or more moieties being analysed or being used in analysis. This can be done by assigning it spatial co-ordinates, which may be provided, stored or processed or provided by a computer. In some cases the location may be determined by a sensor (e.g. a CCD device), which may be operatively linked with a computer.

DNA VACCINES

Any of the Fve nucleic acids disclosed here may be administered to an individual in the form of a DNA vaccine. DNA vaccines are known in the art, and are described in detail in, for example, WO03012117, WO03007986, etc.

The Fve may be administered to an individual in the form of a DNA vaccine. A DNA encoding the Fve, for example, a Fve nucleic acid as disclosed here, may be in any form, for example in the form of a cloned plasmid DNA or a synthetic oligonucleotide. The DNA may be delivered together with a cytokine, for example, IL-2, and / or other co-stimulatory molecules. The cytokines and / or co-stimulatory molecules may themselves be delivered in the form of plasmid or oligonucleotie DNA.

The response to a DNA vaccine has been shown to be increased by the presence of immunostimulatory DNA sequences (ISS). These can take the form of hexameric motifs containing methylated CpG, according to the formula: 5' purine-purine-CG-pyrimidine-pyrimidine-3'. The DNA vaccines may incorporate these or other ISSs, in the DNA encoding the Fve, in the DNA encoding the cytokine or other co-stimulatory molecules, or in both. A review of the advantages of DNA vaccination is provided by Tighe et al (1998, Immunology Today, 19(2), 89-97).

ANTIBODIES

We also provide monoclonal or polyclonal antibodies to polypeptides or fragments thereof. Thus, we further provide a process for the production of monoclonal or polyclonal antibodies to an Fve polypeptide, fragment, homologue, variant or derivative thereof

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide bearing an epitope(s) from a polypeptide. Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an epitope from a polypeptide contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, we also provide polypeptides or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against epitopes in the polypeptides can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against epitopes in the polypeptides can be screened for various properties; i.e., for isotype and epitope affinity.

An alternative technique involves screening phage display libraries where, for example the phage express scFv fragments on the surface of their coat with a large variety of complementarity determining regions (CDRs). This technique is well known in the art.

Antibodies, both monoclonal and polyclonal, which are directed against epitopes from polypeptides are particularly useful in diagnosis, and those which are neutralising are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies. Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the agent against which protection is desired.

Techniques for raising anti-idiotype antibodies are known in the art. These anti-idiotype antibodies may also be useful in therapy.

For the purposes of this document, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies (scFv). Furthermore, the antibodies and fragments thereof may be humanised antibodies, for example as described in EP-A-239400.

Antibodies may be used in method of detecting polypeptides present in biological samples by a method which comprises: (a) providing an antibody; (b) incubating a biological sample with said antibody under conditions which allow for the formation of an

antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said antibody is formed.

Suitable samples include extracts tissues such as brain, breast, ovary, lung, colon, pancreas, testes, liver, muscle and bone tissues or from neoplastic growths derived from such tissues.

Antibodies may be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

ASSAYS

We disclose assays that are suitable for identifying substances which bind to Fve polypeptides, or fragments, homologues, variants or derivatives thereof

In general, such binding assays involve exposing a Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof to a candidate molecule and detecting an interaction or binding between the Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof and the candidate molecule. The binding assay may be conducted *in vitro*, or *in vivo*.

We disclose assays for identifying substances which are capable of potentiating the activities of Fve polypeptide. Activities of Fve have been described in detail above. Such compounds may be employed as agonists of Fve polypeptide, and may for example be co-administered to an individual to enhance any desired effect.

In general, an assay to identify such substances or compounds involves providing a cell or organism, exposing the cell or organism to a Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof, exposing the cell to a candidate molecule, and detecting an effect associated with Fve. Any Fve polypeptide mediated

effect or funciton, as disclosed in this document, particularly the Examples, may be detected.

In particular, the Fve polypeptide mediated effect is preferably chosen from the group consisting of: up-regulation of expression of Th1 cytokines, preferably IFN-γ and TNF-α, down-regulation of expression of Th2 cytokines, preferably IL-4 and IL-13, hemagglutination activity, cell aggregation activity, lymphocyte aggregation activity, lymphoproliferation activity, up-regulation of expression of IL-2, IFN-γ, TNF-α, but not IL-4 in $CD3^+$ T cells, interaction with T and NK cells, adjuvant activity, stimulation of $CD3^+$ $CD16^+$ $CD56^+$ natural killer (NK) T cells, up-regulation of expression of allergen specific IgG2a antibody, prevention of systemic anaphylactic reactions and/or descreased footpad edema, prefereably as assayed using the Arthus reaction (Ko et al, 1995).

In order to identify agonists, an additive or preferably synergistic effect is detected. Thus, while Fve polypeptide on its own is, for example, capable of reducing a level or number, or down-regulation of expression of a molecule, the assays identify molecules which further reduce the level, number or further down-regulate the expression of a molecule. Thus, preferably, the candidate molecule in conjunction with the Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof, down-regulates the expression of, or reduces the level or number, by more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, or more compared to an Fve polypeptide on its own. Thus, for example, a candidate molecule suitable for use as an agonist is one which is capable of enhancing by 10% more the up-regulation of expression of Th1 cytokines, preferably IFN-γ and TNF-α, achieved by Fve polypeptide on its own.

Conversely, assays to identify antagonists involve the detection of a reduction in Fve polypeptide mediated effect. Preferably, the down-regulation of expression or reduction in number or level achieved by Fve polypeptide is reduced in the presence of a suitable candidate molecule. Preferably, the reduction is at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably

at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, or more compared to an Fve polypeptide on its own. Thus, for example, a candidate molecule suitable for use as an antagonist is one which is capable of reducing by 10% more the up-regulation of expression of Th1 cytokines, preferably IFN-γ and TNF-α, achieved by Fve polypeptide on its own.

As an illustration, if N1 is the expression of Th1 cytokines, in an untreated organism or cell, and N2 the expression in an organism or cell exposed to Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof, the expression of Th1 cytokines is increased by R = (N2-N1)/N1 x 100%. Agonists increase R, by a factor x, where x is greater than 1 (e.g., x = 1, 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, 5, 10, 20, 50, 100 etc); while antagonists decrease R, by a factor x, where x is less than 1 (e.g., x = 0.9, 0.9, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 etc).

For example, an organism may be exposed to a Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof and a candidate molecule, and any of the biological activities as set out above, or any combination, detected. Preferred candidate molecules are those which provide an additive or synergistic effect in combination with Fve.

Also disclosed are assays to identify antagonists of Fve polypeptide. Such assays involve detecting a reduced effect on exposure of a cell or organism to an Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof in conjunction with a candidate molecule.

In a preferred embodiment, the assays are conducted on whole organisms rather than cells. Preferably, the organism is one which suffers from a disease as disclosed in this document, or exhibits one or more symptoms of such a disease.

CANDIDATE MOLECULES

Suitable candidate molecules for use in the above assays include peptides, especially of from about 5 to 30 or 10 to 25 amino acids in size. Peptides from panels of peptides comprising random sequences or sequences which have been varied consistently to provide a maximally diverse panel of peptides may be used.

Suitable candidate molecules also include antibody products (for example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies). Furthermore, combinatorial libraries, peptide and peptide mimetics, defined chemical entities, oligonucleotides, and natural product libraries may be screened for activity. The candidate molecules may be used in an initial screen in batches of, for example 10 types of molecules per reaction, and the molecules of those batches which show enhancement or reduction of a Fve polypeptide mediated effect tested individually.

LIBRARIES

Libraries of candidate molecules, such as libraries of polypeptides or nucleic acids, may be employed in the methods and compositions described here. Such libraries are exposed a cell or organism in the presence of a Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof, and an Fve polypeptide mediated effect detected and assayed as described above.

Selection protocols for isolating desired members of large libraries are known in the art, as typified by phage display techniques. Such systems, in which diverse peptide sequences are displayed on the surface of filamentous bacteriophage (Scott and Smith (1990 *supra*), have proven useful for creating libraries of antibody fragments (and the nucleotide sequences that encoding them) for the *in vitro* selection and amplification of specific antibody fragments that bind a target antigen. The nucleotide sequences encoding the V_H and V_L regions are linked to gene fragments which encode leader signals that direct

them to the periplasmic space of *E. coli* and as a result the resultant antibody fragments are displayed on the surface of the bacteriophage, typically as fusions to bacteriophage coat proteins (e.g., pIII or pVIII). Alternatively, antibody fragments are displayed externally on lambda phage capsids (phagebodies). An advantage of phage-based display systems is that, because they are biological systems, selected library members can be amplified simply by growing the phage containing the selected library member in bacterial cells. Furthermore, since the nucleotide sequence that encodes the polypeptide library member is contained on a phage or phagemid vector, sequencing, expression and subsequent genetic manipulation is relatively straightforward.

Methods for the construction of bacteriophage antibody display libraries and lambda phage expression libraries are well known in the art (McCafferty *et al.* (1990) **supra**; Kang *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.*, 88: 4363; Clackson *et al.* (1991) *Nature*, 352: 624; Lowman *et al.* (1991) *Biochemistry*, 30: 10832; Burton *et al.* (1991) *Proc. Natl. Acad. Sci U.S.A.*, 88: 10134; Hoogenboom *et al.* (1991) Nucleic Acids Res., 19: 4133; Chang *et al.* (1991) *J. Immunol.*, 147: 3610; Breitling *et al.* (1991) *Gene*, 104: 147; Marks *et al.* (1991) supra; Barbas *et al.* (1992) supra; Hawkins and Winter (1992) *J. Immunol.*, 22: 867; Marks *et al.*, 1992, *J. Biol. Chem.*, 267: 16007; Lerner *et al.* (1992) *Science*, 258: 1313, incorporated herein by reference). Such techniques may be modified if necessary for the expression generally of polypeptide libraries.

One particularly advantageous approach has been the use of scFv phage-libraries (Bird, R.E., *et al.* (1988) *Science* **242**: 423-6, Huston *et al.*, 1988, Proc. Natl. Acad. Sci U.S.A., 85: 5879-5883; Chaudhary *et al.* (1990) Proc. Natl. Acad. Sci U.S.A., 87: 1066-1070; McCafferty *et al.* (1990) supra; Clackson *et al.* (1991) supra; Marks *et al.* (1991) supra; Chiswell *et al.* (1992) Trends Biotech., 10: 80; Marks *et al.* (1992) supra). Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described. Refinements of phage display approaches are also known, for example as described in WO96/06213 and WO92/01047 (Medical Research Council *et al.*) and WO97/08320 (Morphosys, supra), which are incorporated herein by reference.

Alternative library selection technologies include bacteriophage lambda expression systems, which may be screened directly as bacteriophage plaques or as colonies of lysogens, both as previously described (Huse *et al.* (1989) *Science*, 246: 1275; Caton and Koprowski (1990) *Proc. Natl. Acad. Sci. U.S.A.*, 87; Mullinax *et al.* (1990) *Proc. Natl. Acad. Sci. U.S.A.*, 87: 8095; Persson *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.*, 88: 2432) and are of use. These expression systems may be used to screen a large number of different members of a library, in the order of about 10^6 or even more. Other screening systems rely, for example, on direct chemical synthesis of library members. One early method involves the synthesis of peptides on a set of pins or rods, such as described in WO84/03564. A similar method involving peptide synthesis on beads, which forms a peptide library in which each bead is an individual library member, is described in U.S. Patent No. 4,631,211 and a related method is described in WO92/00091. A significant improvement of the bead-based methods involves tagging each bead with a unique identifier tag, such as an oligonucleotide, so as to facilitate identification of the amino acid sequence of each library member. These improved bead-based methods are described in WO93/06121.

Another chemical synthesis method involves the synthesis of arrays of peptides (or peptidomimetics) on a surface in a manner that places each distinct library member (e.g., unique peptide sequence) at a discrete, predefined location in the array. The identity of each library member is determined by its spatial location in the array. The locations in the array where binding interactions between a predetermined molecule (e.g., a receptor) and reactive library members occur is determined, thereby identifying the sequences of the reactive library members on the basis of spatial location. These methods are described in U.S. Patent No. 5,143,854; WO90/15070 and WO92/10092; Fodor *et al.* (1991) *Science*, 251: 767; Dower and Fodor (1991) *Ann. Rep. Med. Chem.*, 26: 271.

Other systems for generating libraries of polypeptides or nucleotides involve the use of cell-free enzymatic machinery for the *in vitro* synthesis of the library members. In one method, RNA molecules are selected by alternate rounds of selection against a target ligand and PCR amplification (Tuerk and Gold (1990) *Science*, 249: 505; Ellington and Szostak (1990) *Nature*, 346: 818). A similar technique may be used to identify DNA

sequences which bind a predetermined human transcription factor (Thiesen and Bach (1990) *Nucleic Acids Res.*, 18: 3203; Beaudry and Joyce (1992) *Science*, 257: 635; WO92/05258 and WO92/14843). In a similar way, *in vitro* translation can be used to synthesise polypeptides as a method for generating large libraries. These methods which generally comprise stabilised polysome complexes, are described further in WO88/08453, WO90/05785, WO90/07003, WO91/02076, WO91/05058, and WO92/02536. Alternative display systems which are not phage-based, such as those disclosed in WO95/22625 and WO95/11922 (Affymax) use the polysomes to display polypeptides for selection. These and all the foregoing documents also are incorporated herein by reference.

COMBINATORIAL LIBRARIES

Libraries, in particular, libraries of candidate molecules, may suitably be in the form of combinatorial libraries (also known as combinatorial chemical libraries).

A "combinatorial library", as the term is used in this document, is a collection of multiple species of chemical compounds that consist of randomly selected subunits. Combinatorial libraries may be screened for molecules which are capable of potentiating, enhancing, reducing or minimising the a Fve polypeptide mediated effect when exposed to a cell or organism.

Various combinatorial libraries of chemical compounds are currently available, including libraries active against proteolytic and non-proteolytic enzymes, libraries of agonists and antagonists of G-protein coupled receptors (GPCRs), libraries active against non-GPCR targets (e.g., integrins, ion channels, domain interactions, nuclear receptors, and transcription factors) and libraries of whole-cell oncology and anti-infective targets, among others. A comprehensive review of combinatorial libraries, in particular their construction and uses is provided in Dolle and Nelson (1999), *Journal of Combinatorial Chemistry*, Vol 1 No 4, 235-282. Reference is also made to *Combinatorial peptide library protocols* (edited by Shmuel Cabilly, Totowa, N.J.: Humana Press, c1998. *Methods in Molecular Biology*; v. 87).

Further references describing chemical combinatorial libraries, their production and use include those available from the URL http://www.netsci.org/Science/Combichem/, including The Chemical Generation of Molecular Diversity. Michael R. Pavia, Sphinx Pharmaceuticals, A Division of Eli Lilly (Published July, 1995); Combinatorial Chemistry: A Strategy for the Future - MDL Information Systems discusses the role its Project Library plays in managing diversity libraries (Published July, 1995); Solid Support Combinatorial Chemistry in Lead Discovery and SAR Optimization, Adnan M. M. Mjalli and Barry E. Toyonaga, Ontogen Corporation (Published July, 1995); Non-Peptidic Bradykinin Receptor Antagonists From a Structurally Directed Non-Peptide Library. Sarvajit Chakravarty, Babu J. Mavunkel, Robin Andy, Donald J. Kyle*, Scios Nova Inc. (Published July, 1995); Combinatorial Chemistry Library Design using Pharmacophore Diversity Keith Davies and Clive Briant, Chemical Design Ltd. (Published July, 1995); A Database System for Combinatorial Synthesis Experiments - Craig James and David Weininger, Daylight Chemical Information Systems, Inc. (Published July, 1995); An Information Management Architecture for Combinatorial Chemistry, Keith Davies and Catherine White, Chemical Design Ltd. (Published July, 1995); Novel Software Tools for Addressing Chemical Diversity, R. S. Pearlman, Laboratory for Molecular Graphics and Theoretical Modeling, College of Pharmacy, University of Texas (Published June/July, 1996); Opportunities for Computational Chemists Afforded by the New Strategies in Drug Discovery: An Opinion, Yvonne Connolly Martin, Computer Assisted Molecular Design Project, Abbott Laboratories (Published June/July, 1996); Combinatorial Chemistry and Molecular Diversity Course at the University of Louisville: A Description, Arno F. Spatola, Department of Chemistry, University of Louisville (Published June/July, 1996); Chemically Generated Screening Libraries: Present and Future. Michael R. Pavia, Sphinx Pharmaceuticals, A Division of Eli Lilly (Published June/July, 1996); Chemical Strategies For Introducing Carbohydrate Molecular Diversity Into The Drug Discovery Process.. Michael J. Sofia, Transcell Technologies Inc. (Published June/July, 1996); Data Management for Combinatorial Chemistry. Maryjo Zaborowski, Chiron Corporation and Sheila H. DeWitt, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company (Published November, 1995); and The Impact of High Throughput Organic Synthesis on R&D in Bio-Based Industries, John P. Devlin (Published March, 1996).

Techniques in combinatorial chemistry are gaining wide acceptance among modern methods for the generation of new pharmaceutical leads (Gallop, M. A. et al., 1994, J. Med. Chem. 37:1233-1251; Gordon, E. M. et al., 1994, J. Med. Chem. 37:1385-1401.). One combinatorial approach in use is based on a strategy involving the synthesis of libraries containing a different structure on each particle of the solid phase support, interaction of the library with a soluble receptor, identification of the `bead` which interacts with the macromolecular target, and determination of the structure carried by the identified `bead` (Lam, K. S. et al., 1991, Nature 354:82-84). An alternative to this approach is the sequential release of defined aliquots of the compounds from the solid support, with subsequent determination of activity in solution, identification of the particle from which the active.compound was released, and elucidation of its structure by direct sequencing (Salmon, S. E. et al., 1993, Proc.Natl.Acad.Sci.USA 90:11708-11712), or by reading its code (Kerr, J. M. et al., 1993, J.Am.Chem.Soc. 115:2529-2531; Nikolaiev, V. et al., 1993, Pept. Res. 6:161-170; Ohlmeyer, M. H. J. et al., 1993, Proc.Natl.Acad.Sci.USA 90:10922-10926).

Soluble random combinatorial libraries may be synthesized using a simple principle for the generation of equimolar mixtures of peptides which was first described by Furka (Furka, A. et al., 1988, Xth International Symposium on Medicinal Chemistry, Budapest 1988; Furka, A. et al., 1988, 14th International Congress of Biochemistry, Prague 1988; Furka, A. et al., 1991, Int. J. Peptide Protein Res. 37:487-493). The construction of soluble libraries for iterative screening has also been described (Houghten, R. A. et al.1991, Nature 354:84-86). K. S. Lam disclosed the novel and unexpectedly powerful technique of using insoluble random combinatorial libraries. Lam synthesized random combinatorial libraries on solid phase supports, so that each support had a test compound of uniform molecular structure, and screened the libraries without prior removal of the test compounds from the support by solid phase binding protocols (Lam, K. S. et al., 1991, Nature 354:82-84).

Thus, a library of candidate molecules may be a synthetic combinatorial library (e.g., a combinatorial chemical library), a cellular extract, a bodily fluid (e.g., urine, blood,

tears, sweat, or saliva), or other mixture of synthetic or natural products (e.g., a library of small molecules or a fermentation mixture).

A library of molecules may include, for example, amino acids, oligopeptides, polypeptides, proteins, or fragments of peptides or proteins; nucleic acids (e.g., antisense; DNA; RNA; or peptide nucleic acids, PNA); aptamers; or carbohydrates or polysaccharides. Each member of the library can be singular or can be a part of a mixture (e.g., a compressed library). The library may contain purified compounds or can be "dirty" (i.e., containing a significant quantity of impurities). Commercially available libraries (e.g., from Affymetrix, ArQule, Neose Technologies, Sarco, Ciddco, Oxford Asymmetry, Maybridge, Aldrich, Panlabs, Pharmacopoeia, Sigma, or Tripose) may also be used with the methods described here.

In addition to libraries as described above, special libraries called diversity files can be used to assess the specificity, reliability, or reproducibility of the new methods. Diversity files contain a large number of compounds (e.g., 1000 or more small molecules) representative of many classes of compounds that could potentially result in nonspecific detection in an assay. Diversity files are commercially available or can also be assembled from individual compounds commercially available from the vendors listed above.

CANDIDATE SUBSTANCES

Suitable candidate substances include peptides, especially of from about 5 to 30 or 10 to 25 amino acids in size, based on the sequence of the polypeptides described in the Examples, or variants of such peptides in which one or more residues have been substituted. Peptides from panels of peptides comprising random sequences or sequences which have been varied consistently to provide a maximally diverse panel of peptides may be used.

Suitable candidate substances also include antibody products (for example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and

CDR-grafted antibodies) which are specific for a polypeptide. Furthermore, combinatorial libraries, peptide and peptide mimetics, defined chemical entities, oligonucleotides, and natural product libraries may be screened for activity as inhibitors of binding of a polypeptide to the cell division cycle machinery, for example mitotic/meiotic apparatus (such as microtubules). The candidate substances may be used in an initial screen in batches of, for example 10 substances per reaction, and the substances of those batches which show inhibition tested individually. Candidate substances which show activity in *in vitro* screens such as those described below can then be tested in whole cell systems, such as mammalian cells which will be exposed to the inhibitor and tested for inhibition of any of the stages of the cell cycle.

POLYPEPTIDE BINDING ASSAYS

One type of assay for identifying substances that bind to a polypeptide involves contacting a polypeptide, which is immobilised on a solid support, with a non-immobilised candidate substance determining whether and/or to what extent the polypeptide and candidate substance bind to each other. Alternatively, the candidate substance may be immobilised and the polypeptide non-immobilised. This may be used to detect substances capable of binding to Fve polypeptides, or fragments, homologues, variants or derivatives thereof.

In a preferred assay method, the polypeptide is immobilised on beads such as agarose beads. Typically this is achieved by expressing the Fve polypeptide, or a fragment, homologue, variant or derivative thereof as a GST-fusion protein in bacteria, yeast or higher eukaryotic cell lines and purifying the GST-fusion protein from crude cell extracts using glutathione-agarose beads (Smith and Johnson, 1988). As a control, binding of the candidate substance, which is not a GST-fusion protein, to the immobilised polypeptide is determined in the absence of the polypeptide. The binding of the candidate substance to the immobilised polypeptide is then determined. This type of assay is known in the art as a GST pulldown assay. Again, the candidate substance may be immobilised and the polypeptide non-immobilised.

It is also possible to perform this type of assay using different affinity purification systems for immobilising one of the components, for example Ni-NTA agarose and histidine-tagged components.

Binding of the Fve polypeptide, or a fragment, homologue, variant or derivative thereof to the candidate substance may be determined by a variety of methods well-known in the art. For example, the non-immobilised component may be labeled (with for example, a radioactive label, an epitope tag or an enzyme-antibody conjugate). Alternatively, binding may be determined by immunological detection techniques. For example, the reaction mixture can be Western blotted and the blot probed with an antibody that detects the non-immobilised component. ELISA techniques may also be used.

Candidate substances are typically added to a final concentration of from 1 to 1000 nmol/ml, more preferably from 1 to 100 nmol/ml. In the case of antibodies, the final concentration used is typically from 100 to 500 μg/ml, more preferably from 200 to 300 μg/ml.

FVE DISEASES

As disclosed elsewhere in this document, Fve polypeptides, nucleic acids, and fragments, homologues, variants and derivatives thereof, host cells, vectors, DNA vaccines, etc, are suitable for treating or preventing various diseases (here referred to as "Fve diseases"). They may be be administered in an amount in the range of 1 microgram to 1 gramme to an average human patient or individual to be vaccinated. It is preferred to use a smaller dose in the ragne of 1 microgram to 1 milligram for each administration, however.

The Fve polypeptides, etc may be adminisstered togeher, either simultaneously or separately with compounds such as cytokines and / or or growth factors, such as interleukin-2 (IL-2), Interleukin 12 (IL-12), GM-CSF or the like in order to strenghthen the immune response. The Fve polypeptides, etc can be used in a vaccine or a therapeutic

composition either alone or in combination with other materials, for example, in the form of a lipopeptide conjugate which is known to induce a high-affinity cytotoxic T cell responses (Deres, 1989, Nature 342).

In particular, Fve diseases include allergies and cancer, described in further detail below.

Cancer

Fve polypeptides, nucleic acids, and fragments, homologues, variants and derivatives thereof, are suitable for treating or preventing cancer.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer. Further examples are solid tumor cancer including colon cancer, breast cancer, lung cancer and prostrate cancer, hematopoietic malignancies including leukemias and lymphomas, Hodgkin's disease, aplastic anemia, skin cancer and familiar adenomatous polyposis. Further examples include brain neoplasms, colorectal neoplasms, breast neoplasms, cervix neoplasms, eye neoplasms, liver neoplasms, lung neoplasms, pancreatic neoplasms, ovarian neoplasms, prostatic neoplasms, skin neoplasms, testicular neoplasms, neoplasms, bone neoplasms, yellow fevertrophoblastic neoplasms, fallopian tube neoplasms, rectal neoplasms, colonic neoplasms, kidney neoplasms, stomach neoplasms, and parathyroid neoplasms. Breast cancer, prostate cancer, pancreatic cancer, colorectal cancer, lung cancer, malignant melanoma, leukaemia, lympyhoma, ovarian cancer, cervical cancer and biliary tract carcinoma are also included.

In preferred embodiments, Fve polypeptide, nucleic acid, and fragments, homologues, variants and derivatives thereof are used to treat T cell lymphoma, melanoma or lung cancer.

The Fve polypeptides and nucleic acids, etc, as described here, may also be used in combination with anticancer agents such as endostatin and angiostatin or cytotoxic agents or chemotherapeutic agent. For example, drugs such as such as adriamycin, daunomycin, cis-platinum, etoposide, taxol, taxotere and alkaloids, such as vincristine, and antimetabolites such as methotrexate. The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. I, Y, Pr), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

Also, the term includes oncogene product/tyrosine kinase inhibitors, such as the bicyclic ansamycins disclosed in WO 94/22867; 1,2-bis(arylamino) benzoic acid derivatives disclosed in EP 600832; 6,7-diamino-phthalazin-1-one derivatives disclosed in EP 600831; 4,5-bis(arylamino)-phthalimide derivatives as disclosed in EP 516598; or peptides which inhibit binding of a tyrosine kinase to a SH2-containing substrate protein (see WO 94/07913, for example). A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Adriamycin, Doxorubicin, 5-Fluorouracil (5-FU), Cytosine arabinoside (Ara-C), Cyclophosphamide, Thiotepa, Busulfan, Cytoxin, Taxol, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincristine, VP-16, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Nicotinamide, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan and other related nitrogen mustards, and endocrine therapies (such as diethylstilbestrol (DES), Tamoxifen, LHRH antagonizing drugs, progestins, anti-progestins etc).

Allergies

Existing treatments for allergies typically involve the long-term use of steroids to depress the immune system. There are undesirable side effects with long-term steroid therapy. We demonstrate that Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof (as well as DNA vaccines, host cells and transgenic organisms comprising any of these) may be used to alleviate the symptoms of allergy, or to treat allergy. The term "allergy" as used here, refers to any allergic reactions such as allergic contact hypersensitivity.

In general, the allergy may be to an allergen from any source, for example, a source known to induce allergenic responses in humans. For example, the allergy may be to a tree pollen allergen, a grass pollen allergen, a weed pollen allergen, a feline antigen, or a fungal allergen. Thus, the allergy may be to a tree pollen allergen, for example Bet v 1 and Bet v 2 from birch tree. The allergy may be to a grass pollen allergen, for example, Phl p 1 and Phl p 2 from timothy grass. It may be to a weed pollen allergen, for example, antigen E

from ragweed. It may be to an animal allergen, for example, a canine or feline antigen. Specifically, it may be to a major feline antigen, for example, Fel d 1. The allergy may be to a fungal allergen, for example a major fungal allergen, for example, Asp f1, Asp f2, and Asp f3 from *Aspergillus fumigatus*.

In preferred embodiments, the allergy is to a dust mite allergen, preferably a house dust mite allergen. In particular, the allergen is preferably derived from a mite from Family Glycyphagidae or Family Pyroglyphidae. Dust mites of Family Glycyphagidae include those in the genera Aeroglyphus, Austroglycyphagus, Blomia, Ctenoglyphus, Glycyphagus, Gohieria, Lepidoglyphus. Dust mites of Family Pyroglyphidae include those in the genera Dermatophagoides, Euroglyphus, Pyroglyphus. In preferred embodiments, the allergy is preferably to an allergen from a species in any of these genera.

In highly preferred embodiments, the allergy is to an allergen which is a group 1 allergen (Der p 1, Der f 1, Blo t 1, Eur m1, Lep d 1), a group 2 allergen (Der p 2, Der f 2, Blo t 2, Eur m 2, Lep d 2), a group 5 allergen (Blo t 5, Der p 5, Der f 5, Eur m 5, Lep d 5) or a group 15 allergen (Der p 15, Der f 15, Blot 15, Eur m 15, Lep d 15) from dust mite.

Allergies suitable for treatment with Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof may therefore include a seasonal respiratory allergy, allergic rhinitis, hayfever, nonallergic rhinitis, vasomotor rhinitis, irritant rhinitis, an allergy against grass pollens, tree pollens or animal danders, an allergy associated with allergic asthma, and food allergies. In particular, and as described elsewhere, Fve polypeptide, nucleic acid, or a fragment, homologue, variant or derivative thereof may be used to treat allergies to house dust mite (*Dermatophagoides* spp), preferably *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*, or to fungi or fungal spores, preferably *Aspergillus fumigatus*. Preferably, the allergens are comprised in faeces of *Dermatophagoides spp.*

Viral Infections

The immunomodulator-viral infectious antigen combinations, preferably conjugates, may be used to treat or prevent any of a number of viral infectious diseases. The virus concerned may be an RNA virus or a DNA virus. Preferably, the virus is an integrating virus. Preferably, the virus is selected from a lentivirus and a herpesvirus. More preferably, the virus is an HIV virus or a HSV virus.

The methods described here can therefore be used to prevent the development and establishment of diseases caused by or associated with any of the above viruses, including human immunodeficiency virus, such as HIV-1 and HIV-2, and herpesvirus, for example HSV-1, HSV-2, HSV-7 and HSV-8, as well as human cytomegalovirus, varicella-zoster virus, Epstein-Barr virus and human herpesvirus 6.in humans.

Examples of viruses which may be targeted using the methods and compositions described here are given in the tables below.

DNA VIRUSES

Family	Genus or [Subfamily]	Example	Diseases
Herpesviridae	[Alphaherpes-virinae]	Herpes simplex virus type 1 *(aka HHV-1)*	Encephalitis, cold sores, gingivostomatitis
		Herpes simplex virus type 2 *(aka HHV-2)*	Genital herpes, encephalitis
		Varicella zoster virus *(aka HHV-3)*	Chickenpox, shingles
	[Gammaherpesvirinae]	Epstein Barr virus *(aka HHV-4)*	Mononucleoisis, hepatitis, tumors (BL, NPC)
		Kaposi's sarcoma associated herpesvirus, KSHV *(aka Human herpesvirus 8)*	?Probably: tumors, inc. Kaposi's sarcoma (KS) and some B cell lymphomas
	[Betaherpesvirinae]	Human cytomegalovirus *(aka HHV-5)*	Mononucleosis, hepatitis, pneumonitis, congenital
		Human herpesvirus 6	Roseola (aka E. subitum), pneumonitis
		Human herpesvirus 7	Some cases of roseola?
Adenoviridae	Mastadenovirus	Human adenoviruses	50 serotypes (species); respiratory infections
Papovaviridae	Papillomavirus	Human papillomaviruses	80 species; warts and tumors
	Polyomavirus	JC, BK viruses	Mild usually; JC causes PML in AIDS
Hepadnaviridae	Orthohepadnavirus	Hepatitis B virus (HBV)	Hepatitis (chronic), cirrhosis, liver tumors
		Hepatitis C virus (HCV)	Hepatitis (chronic), cirrhosis, liver tumors
Poxviridae	Orthopoxvirus	Vaccinia virus	Smallpox vaccine virus
		Monkeypox virus	Smallpox-like disease; a rare zoonosis (recent outbreak in Congo; 92 cases from 2/96 - 2/97)
	Parapoxvirus	Orf virus	Skin lesions ("pocks")
Parvoviridae	Erythrovirus	B19 parvovirus	E. infectiousum (aka Fifth disease), aplastic crisis, fetal loss
Circoviridae	Dependovirus	Adeno-associated virus	Useful for gene therapy; integrates into chromosome

	Circovirus	TT virus (TTV)	Linked to hepatitis of unknown etiology

RNA VIRUSES

Family	Genus or [Subfamily]	Example	Diseases
Picornaviridae	Enterovirus	Polioviruses	3 types; Aseptic meningitis, paralytic poliomyelitis
		Echoviruses	30 types; Aseptic meningitis, rashes
		Coxsackieviruses	30 types; Aseptic meningitis, myopericarditis
	Hepatovirus	Hepatitis A virus	Acute hepatitis (fecal-oral spread)
	Rhinovirus	Human rhinoviruses	115 types; Common cold
Caliciviridae	Calicivirus	Norwalk virus	Gastrointestinal illness
Paramyxoviridae	Paramyxovirus	Parainfluenza viruses	4 types; Common cold, bronchiolitis, pneumonia
	Rubulavirus	Mumps virus	Mumps: parotitis, aseptic meningitis (rare: orchitis, encephalitis)
	Morbillivirus	Measles virus	Measles: fever, rash (rare: encephalitis, SSPE)
	Pneumovirus	Respiratory syncytial virus	Common cold (adults), bronchiolitis, pneumonia (infants)
Orthomyxo-viridae	Influenzavirus A	Influenza virus A	Flu: fever, myalgia, malaise, cough, pneumonia
	Influenzavirus B	Influenza virus B	Flu: fever, myalgia, malaise, cough, pneumonia
Rhabdoviridae	Lyssavirus	Rabies virus	Rabies: long incubation, then CNS disease, death
Filoviridae	Filovirus	Ebola and Marburg viruses	Hemorrhagic fever, death
Bornaviridae	Bornavirus	Borna disease virus	Uncertain; linked to schizophrenia-like disease in some animals
Retroviridae	Deltaretrovirus	Human T-lymphotropic virus type-I	Adult T-cell leukemia (ATL), tropical spastic paraparesis (TSP)
	Spumavirus	Human foamy viruses	No disease known
	Lentivirus	Human immunodeficiency virus type-1 and -2	AIDS, CNS disease
Togaviridae	Rubivirus	Rubella virus	Mild exanthem; congenital fetal defects
	Alphavirus	Equine encephalitis viruses (WEE, EEE, VEE)	Mosquito-born, encephalitis
Flaviviridae	Flavivirus	Yellow fever virus	Mosquito-born; fever, hepatitis (yellow fever!)
		Dengue virus	Mosquito-born; hemorrhagic fever
		St. Louis Encephalitis virus	Mosquito-born; encephalitis
	Hepacivirus	Hepatitis C virus	Hepatitis (often chronic), liver cancer
		Hepatitis G virus	*Hepatitis???*
Reoviridae	Rotavirus	Human rotaviruses	Numerous serotypes; Diarrhea
	Coltivirus	Colorado Tick Fever virus	Tick-born; fever
	Orthoreovirus	Human reoviruses	Minimal disease
Bunyaviridae	Hantavirus	Pulmonary Syndrome Hantavirus	Rodent spread; pulmonary illness (can be lethal, "Four Corners" outbreak)
		Hantaan virus	Rodent spread; hemorrhagic fever with renal syndrome
	Phlebovirus	Rift Valley Fever virus	Mosquito-born; hemorrhagic fever
	Nairovirus	Crimean-Congo Hemorrhagic Fever virus	Mosquito-born; hemorrhagic fever
Arenaviridae	Arenavirus	Lymphocytic Choriomeningitis virus	Rodent-born; fever, aseptic meningitis
		Lassa virus	Rodent-born; severe hemorrhagic fever (BL4 agents; also: Machupo, Junin)
	Deltavirus	Hepatitis Delta virus	Requires HBV to grow; hepatitis, liver cancer
Coronaviridae	Coronavirus	Human coronaviruses	Mild common cold-like illness
Astroviridae	Astrovirus	Human astroviruses	Gastroenteritis
Unclassified	"Hepatitis E-like viruses"	Hepatitis E virus	Hepatitis (acute); fecal-oral spread

Human Immunodeficiency Virus-1 (HIV-1)

The combinations and conjugates described here, including Fve polypeptide combinations and conjugates, may be used to treat or prevent Human Immunodeficiency Virus (HIV) infection. The methods described here can therefore be used to prevent the development and establishment of diseases caused by or associated with human immunodeficiency virus, such as HIV-1 and HIV-2.

Human Immunodeficiency Virus (HIV) is a retrovirus which infects cells of the immune system, most importantly $CD4^+$ T lymphocytes. $CD4^+$ T lymphocytes are important, not only in terms of their direct role in immune function, but also in stimulating normal function in other components of the immune system, including $CD8^+$ T-lymphocytes. These HIV infected cells have their function disturbed by several mechanisms and/or are rapidly killed by viral replication. The end result of chronic HIV infection is gradual depletion of $CD4^+$ T lymphocytes, reduced immune capacity, and ultimately the development of AIDS, leading to death.

The regulation of HIV gene expression is accomplished by a combination of both cellular and viral factors. HIV gene expression is regulated at both the transcriptional and post-transcriptional levels. The HIV genes can be divided into the early genes and the late genes. The early genes, Tat, Rev, and Nef, are expressed in a Rev-independent manner. The mRNAs encoding the late genes, Gag, Pol, Env, Vpr, Vpu, and Vif require Rev to be cytoplasmically localized and expressed. HIV transcription is mediated by a single promoter in the 5' LTR. Expression from the 5' LTR generates a 9-kb primary transcript that has the potential to encode all nine HIV genes. The primary transcript is roughly 600 bases shorter than the provirus. The primary transcript can be spliced into one of more than 30 mRNA species or packaged without further modification into virion particles (to serve as the viral RNA genome).

Any of the HIV proteins disclosed here may be used as a viral infectious antigen for productions of conjugates and combinations as described above.

Herpes Virus

The combinations and conjugates described here, including Fve polypeptide combinations and conjugates, may be used to treat or prevent Herpesvirus infection. The methods described here can therefore be used to prevent the development and establishment of diseases caused by or associated with herpesvirus, for example HSV-1, HSV-2, HSV-7 and HSV-8.

Particular examples of herpesvirus include: herpes simplex virus 1 ("HSV-1"), herpes simplex virus 2 ("HSV-2"), human cytomegalovirus ("HCMV"), varicella-zoster virus ("VZV"), Epstein-Barr virus ("EBV"), human herpesvirus 6 ("HHV6"), herpes simplex virus 7 ("HSV-7") and herpes simplex virus 8 ("HSV-8").

Herpesviruses have also been isolated from horses, cattle, pigs (pseudorabies virus ("PSV") and porcine cytomegalovirus), chickens (infectious larygotracheitis), chimpanzees, birds (Marck's disease herpesvirus 1 and 2), turkeys and fish (see "Herpesviridae: A Brief Introduction", Virology, Second Edition, edited by B. N. Fields, Chapter 64, 1787 (1990)).

Herpes simplex viral ("HSV") infection is generally a recurrent viral infection characterized by the appearance on the skin or mucous membranes of single or multiple clusters of small vesicles, filled with clear fluid, on slightly raised inflammatory bases. The herpes simplex virus is a relatively large-sized virus. HSV-2 commonly causes herpes labialis. HSV-2 is usually, though not always, recoverable from genital lesions. Ordinarily, HSV-2 is transmitted venereally.

Diseases caused by varicella-zoster virus (human herpesvirus 3) include varicella (chickenpox) and zoster (shingles). Cytomegalovirus (human herpesvirus 5) is responsible for cytomegalic inclusion disease in infants. There is presently no specific treatment for treating patients infected with cytomegalovirus. Epstein-Barr virus (human herpesvirus 4) is the causative agent of infectious mononucleosis and has been associated with Burkitt's lymphoma and nasopharyngeal carcinoma. Animal herpesviruses which may pose a

problem for humans include B virus (herpesvirus of Old World Monkeys) and Marmoset herpesvirus (herpesvirus of New World Monkeys).

Herpes simplex virus 1 (HSV-1) is a human pathogen capable of becoming latent in nerve cells. Like all the other members of *Herpesviridae* it has a complex architecture and double-stranded linear DNA genome which encodes for variety of viral proteins including DNA pol. and TK.

HSV gene expression proceeds in a sequential and strictly regulated manner and can be divided into at least three phases, termed immediate-early (IE or α), early (β) and late (γ). The cascade of HSV-1 gene expression starts from IE genes, which are expressed immediately after lytic infection begins. The IE proteins regulate the expression of later classes of genes (early and late) as well as their own expression. The product of IE175k (ICP4) gene is critical for HSV-1 gene regulation and ts mutants in this gene are blocked at IE stage of infection.

The IE genes themselves are activated by a virion structural protein VP16 (expressed late in the replicative cycle and incorporated into HSV particle). All 5 IE genes of HSV-1 (IE110k - 2 copies/HSV genome, IE175 - 2 copies/HSV genome, IE68k, IE63k and IE12k) have at least one copy of a conserved promoter/enhancer sequence - **TAATGARAT.** This sequence is recognized by the transactivation complex which consists of; Oct-1, HCF and VP16. The GARAT element is required for efficient transactivation by VP16. This mechanism of gene activation is unique for HSV and despite Oct-1 being a common transcription factor, the Oct-1/HCF/VP16 complex activates specifically only HSV IE genes.

Any of the herpesvirus proteins disclosed here may be used as a viral infectious antigen for productions of conjugates and combinations as described above.

CYTOKINES

In a further embodiment, the Fve polypeptide, nucleic acid, fragment, homologue, variant or derivative thereof is used to modulate cytokine levels in an individual. Preferably, the level of inflammatory cytokines is down-regulated. Examples of inflammatory cytokines include Granulocyte-Macrophage-Colony stimulating factor (GM-CSF), as well as any cytokine that mediates migration of alveolar macrophages into the lung and act to increase cell proliferation.

The term "cytokine" may be used to refer to any of a number of soluble molecules (e.g., glycoproteins) released by cells of the immune system, which act nonenzymatically through specific receptors to regulate immune responses. Cytokines resemble hormones in that they act at low concentrations bound with high affinity to a specific receptor. Preferably, the term "cytokine" refers to a diverse group of soluble proteins and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues.

Particular examples of cytokines which are suitable for use in the methods and compositions described include interleukins, lymphokine, interferon, Colony Stimulating Factors (CSFs) such as Granulocyte-Colony Stimulating Factor (G-CSF), Macrophage-Colony stimulating factor (M-CSF) and Granulocyte-Macrophage-Colony stimulating factor (GM-CSF), GSF, Platelet-Activating Factors (PAF), Tumor Necrosis Factor (TNF).

Thus, interleukins such as IL1, IL2 and IL4, as well as interferons such as IFN-α, IFN-β and IFN-γ are included. Tumour necrosis factors TNF-α (cachetin), TNF-β (lymphotoxin) may also be suitably employed.

Preferred cytokines are those which are capable of recruiting immune responses, for example, stimulation of dendritic cell or cytotoxic T cell activity, or which are capable

of recruiting macrophages to the target site. In a highly preferred embodiment, the cytokine comprises IL-2, GM-CSF or GSF.

CHEMICAL COUPLING

As noted above, the immunomodulator may be coupled to the allergen by a number of methods. Crosslinkers are divided into homobifunctional crosslinkers, containing two identical reactive groups, or heterobifunctional crosslinkers, with two different reactive groups. Heterobifunctional crosslinkers allow sequential conjugations, minimizing polymerization.

Any of the homobifunctional or heterobifunctional crosslinkers presented in the table below may be used to couple the allergen with the immunomodulator to produce an immunomodulator-allergen conjugate.

Homobifunctional

Reagent	Cat. No.	Modified Group	Solubility	Comments	Refs
BMME	442635-Y	-SH	DMF, Acetone	Homobifunctional crosslinker useful for formation of conjugates via thiol groups.	Weston, P.D., et al. 1980. Biochem. Biophys Acta. 612, 40.
BSOCOES	203851-Y	-NH2	Water	Base cleavable crosslinker useful for studying receptors and mapping surface polypeptide antigens on lymphocytes.	Howard, A.D., et al. 1985. J. Biol. Chem.260, 10833.
DSP	322133-Y	-NH2	Water	Thiol cleavable crosslinker used to immobilize proteins on supports containing amino groups.	Lee, W.T., and Conrad, D.H. 1985. J. Immunol.134, 518.
DSS	322131-Y	-NH2	Water	Non-cleavable, membrane impermeable crosslinker widely used for conjugating radiolabeled ligands to cell surface receptors and for detecting conformational changes in membrane proteins.	D'Souza, S.E., et al. 1988. J. Biol. Chem.263, 3943.
EGS	324550-Y	-NH2	DMSO	Hydroxylamine cleavable reagent for crosslinking and reversible immobilization of proteins through their primary amine groups. Useful for studying structure-function relationships.	Geisler, N., et al. 1992. Eur. J. Biochem.206, 841. 14. Moenner, M., et al. 1986. Proc. Natl. Acad. Sci. USA83, 5024.

EGS, Water Soluble	324551-Y	-NH2	Water	Water soluble version of EGS that reacts rapidly with dilute proteins at neutral pH. Crosslinked proteins are readily cleaved with hydroxylamine at pH 8.5 for 3-6 hours, 37°C.	Yanagi, T., et al. 1989. Agric. Biol. Chem.53, 525.
Glutaraldehyde	354400-Y	-OH	Water	Used for crosslinking proteins and polyhydroxy materials. Conjugates haptens to carrier proteins; also used as a tissue fixative.	Harlow, E., and Lane, D. 1988. Antibodies: A Laboratory Manual, Cold Spring Harbor Publications, N.Y., p. 349.
SATA	573100-Y	-NH2	DMSO	Introduces protected thiols via primary amines. When treated with hydroxylamine, yields a free sulhydryl group that can be conjugated to maleimide-modified proteins.	Duncan, R.J.S., et al. 1983. Anal. Biochem.132, 68.

Heterobifunctional

Reagent	Cat. No.	Modified Group	Solubility	Comments	Refs
GMBS	442630-Y	-NH2, -SH	DMSO	Heterobifunctional crosslinker useful for preparing enzyme-antibody conjugates (e.g. -gal-IgG) and for immobilizing enzymes on solid supports.	Kitagwa, T., et al. 1983. J. Biochem.94, 1160.19. Rusin, K.M., et al. 1992. Biosens. Bioelectron.7, 367.
MBS	442625-Y 442626-Y	-NH2, -SH -NH2, -SH	DMSO, Water	Thiol cleavable, heterobifunctional reagent especially useful for preparing peptide-carrier conjugates and conjugating toxins to antibodies.	Green, N., et al. 1982. Cell 28, 477.
PMPI	528250-Y	-SH2, -OH	DMSO, DMF	Used in the preparation of alkaline phosphatase conjugates of estradiol, progesterone, serine-enriched peptides, and vitamin B12.	Aithal, H.N., et al. 1988. J. Immunol. Methods112, 63.
SMCC	573114-Y 573115-Y	-NH2, -SH -NH2, -SH	DMF, AN Acetonitrile Water	Heterobifunctional reagent for enzyme labeling of antibodies and antibody fragments. The cyclohexane bridge provides extra stability to the maleimide group. Ideal reagent for preserving enzyme activity and antibody specificity after coupling.	Annunziato, M.E., et al. 1993. Bioconjugate Chem.4, 212.
SPDP	573112-Y	-NH2, -SH	DMF, AN Acetonitrile	Introduces protected thiol groups to amine groups. Thiolated proteins can be coupled to a second molecule via an iodoacetamide or maleimide group, or to a second pyridyldisulfide	Caruelle, D., et al. 1988. Anal. Biochem.173, 328.

				containing molecule.	

Each of these reagents may be obtained from a number of manufacturers, for example, from Calbiochem (catalogue number in column 2), or Piece Chemical Company.

PHARMACEUTICAL COMPOSITIONS

Fve polypeptides may be produced in large amounts at low cost in a bioactive form, allowing the development of Fve containing formulations by aerosolisation, nebulisation, intranasal or intratracheal administration.

While it is possible for the composition comprising the Fve polypeptide or nucleic acid to be administered alone, it is preferable to formulate the active ingredient as a pharmaceutical formulation. We therefore also disclose pharmaceutical compositions comprising Fve polypeptide or nucleic acid, or a fragment, homologue, variant or derivative thereof. Such pharmaceutical compositions are useful for delivery of Fve polypeptide, nucleic acid, fragment, homologue, variant or derivative thereof to an individual for the treatment or alleviation of symptoms as described.

The composition may include the Fve polypeptide, nucleic acid, fragment, homologue, variant or derivative thereof, a structurally related compound, or an acidic salt thereof. The pharmaceutical formulations comprise an effective amount of Fve polypeptide, nucleic acid, fragment, homologue, variant or derivative thereof, together with one or more pharmaceutically-acceptable carriers. An "effective amount" of an Fve polypeptide, nucleic acid fragment, homologue, variant or derivative thereof is the amount sufficient to alleviate at least one symptom of a disease as described.

The effective amount will vary depending upon the particular disease or syndrome to be treated or alleviated, as well as other factors including the age and weight of the patient, how advanced the disease etc state is, the general health of the patient, the severity of the symptoms, and whether the Fve polypeptide, nucleic acid, fragment, homologue,

variant or derivative thereof is being administered alone or in combination with other therapies.

Suitable pharmaceutically acceptable carriers are well known in the art and vary with the desired form and mode of administration of the pharmaceutical formulation. For example, they can include diluents or excipients such as fillers, binders, wetting agents, disintegrators, surface-active agents, lubricants and the like. Typically, the carrier is a solid, a liquid or a vaporizable carrier, or a combination thereof. Each carrier should be "acceptable" in the sense of being compatible with the other ingredients in the formulation and not injurious to the patient. The carrier should be biologically acceptable without eliciting an adverse reaction (e.g. immune response) when administered to the host.

The pharmaceutical compositions disclosed here include those suitable for topical and oral administration, with topical formulations being preferred where the tissue affected is primarily the skin or epidermis (for example, psoriasis, eczema and other epidermal diseases). The topical formulations include those pharmaceutical forms in which the composition is applied externally by direct contact with the skin surface to be treated. A conventional pharmaceutical form for topical application includes a soak, an ointment, a cream, a lotion, a paste, a gel, a stick, a spray, an aerosol, a bath oil, a solution and the like. Topical therapy is delivered by various vehicles, the choice of vehicle can be important and generally is related to whether an acute or chronic disease is to be treated. Other formulations for topical application include shampoos, soaps, shake lotions, and the like, particularly those formulated to leave a residue on the underlying skin, such as the scalp (Arndt et al, in Dermatology In General Medicine 2:2838 (1993)).

In general, the concentration of the Fve polypeptide, nucleic acid, fragment, homologue, variant or derivative thereof composition in the topical formulation is in an amount of about 0.5 to 50% by weight of the composition, preferably about 1 to 30%, more preferably about 2-20%, and most preferably about 5-10%. The concentration used can be in the upper portion of the range initially, as treatment continues, the concentration can be lowered or the application of the formulation may be less frequent. Topical

applications are often applied twice daily. However, once-daily application of a larger dose or more frequent applications of a smaller dose may be effective. The stratum corneum may act as a reservoir and allow gradual penetration of a drug into the viable skin layers over a prolonged period of time.

In a topical application, a sufficient amount of active ingredient must penetrate a patient's skin in order to obtain a desired pharmacological effect. It is generally understood that the absorption of drug into the skin is a function of the nature of the drug, the behaviour of the vehicle, and the skin. Three major variables account for differences in the rate of absorption or flux of different topical drugs or the same drug in different vehicles; the concentration of drug in the vehicle, the partition coefficient of drug between the stratum corneum and the vehicle and the diffusion coefficient of drug in the stratum corneum. To be effective for treatment, a drug must cross the stratum corneum which is responsible for the barrier function of the skin. In general, a topical formulation which exerts a high *in vitro* skin penetration is effective *in vivo*. Ostrenga et al (J. Pharm. Sci., 60:1175-1179 (1971) demonstrated that *in vivo* efficacy of topically applied steroids was proportional to the steroid penetration rate into dermatomed human skin *in vitro*.

A skin penetration enhancer which is dermatologically acceptable and compatible with the agent can be incorporated into the formulation to increase the penetration of the active compound(s) from the skin surface into epidermal keratinocytes. A skin enhancer which increases the absorption of the active compound(s) into the skin reduces the amount of agent needed for an effective treatment and provides for a longer lasting effect of the formulation. Skin penetration enhancers are well known in the art. For example, dimethyl sulfoxide (U.S. Pat. No. 3,711,602); oleic acid, 1,2-butanediol surfactant (Cooper, J. Pharm. Sci., 73:1153-1156 (1984)); a combination of ethanol and oleic acid or oleyl alcohol (EP 267,617), 2-ethyl-1,3-hexanediol (WO 87/03490); decyl methyl sulphoxide and Azone.RTM. (Hadgraft, Eur. J. Drug. Metab. Pharmacokinet, 21:165-173 (1996)); alcohols, sulphoxides, fatty acids, esters, Azone.RTM., pyrrolidones, urea and polyoles (Kalbitz et al, Pharmazie, 51:619-637 (1996));

Terpenes such as 1,8-cineole, menthone, limonene and nerolidol (Yamane, J. Pharmacy & Pharmocology, 47:978-989 (1995)); Azone.RTM. and Transcutol (Harrison et al, Pharmaceutical Res. 13:542-546 (1996)); and oleic acid, polyethylene glycol and propylene glycol (Singh et al, Pharmazie, 51:741-744 (1996)) are known to improve skin penetration of an active ingredient.

Levels of penetration of an agent or composition can be determined by techniques known to those of skill in the art. For example, radiolabeling of the active compound, followed by measurement of the amount of radiolabeled compound absorbed by the skin enables one of skill in the art to determine levels of the composition absorbed using any of several methods of determining skin penetration of the test compound. Publications relating to skin penetration studies include Reinfenrath, W G and G S Hawkins. The Weaning Yorkshire Pig as an Animal Model for Measuring Percutaneous Penetration. In:Swine in Biomedical Research (M. E. Tumbleson, Ed.) Plenum, New York, 1986, and Hawkins, G. S. Methodology for the Execution of *In Vitro* Skin Penetration Determinations. In: Methods for Skin Absorption, B W Kemppainen and W G Reifenrath, Eds., CRC Press, Boca Raton, 1990, pp.67-80; and W. G. Reifenrath, Cosmetics & Toiletries, 110:3-9 (1995).

For some applications, it is preferable to administer a long acting form of agent or composition using formulations known in the arts, such as polymers. The agent can be incorporated into a dermal patch (Junginger, H. E., in Acta Pharmaceutica Nordica 4:117 (1992); Thacharodi et al, in Biomaterials 16:145-148 (1995); Niedner R., in Hautarzt 39:761-766 (1988)) or a bandage according to methods known in the arts, to increase the efficiency of delivery of the drug to the areas to be treated.

Optionally, the topical formulations can have additional excipients for example; preservatives such as methylparaben, benzyl alcohol, sorbic acid or quaternary ammonium compound; stabilizers such as EDTA, antioxidants such as butylated hydroxytoluene or butylated hydroxanisole, and buffers such as citrate and phosphate.

The pharmaceutical composition can be administered in an oral formulation in the form of tablets, capsules or solutions. An effective amount of the oral formulation is administered to patients 1 to 3 times daily until the symptoms of the disease alleviated. The effective amount of agent depends on the age, weight and condition of a patient. In general, the daily oral dose of agent is less than 1200 mg, and more than 100 mg. The preferred daily oral dose is about 300-600 mg. Oral formulations are conveniently presented in a unit dosage form and may be prepared by any method known in the art of pharmacy. The composition may be formulated together with a suitable pharmaceutically acceptable carrier into any desired dosage form. Typical unit dosage forms include tablets, pills, powders, solutions, suspensions, emulsions, granules, capsules, suppositories. In general, the formulations are prepared by uniformly and intimately bringing into association the agent composition with liquid carriers or finely divided solid carriers or both, and as necessary, shaping the product. The active ingredient can be incorporated into a variety of basic materials in the form of a liquid, powder, tablets or capsules to give an effective amount of active ingredient to treat the disease.

Other therapeutic agents suitable for use herein are any compatible drugs that are effective for the intended purpose, or drugs that are complementary to the agent formulation. The formulation utilized in a combination therapy may be administered simultaneously, or sequentially with other treatment, such that a combined effect is achieved.

The invention is described further, for the purpose of illustration only, in the following examples.

EXAMPLES

In each of the Examples presented below, where an activity is described for a Fve polypeptide comprising a GST (glutathione S transferase) portion (for example, as a GST-FIP fusion protein), we find that the polypeptide itself, without the GST portion, has

substantially the same activity. This is to be expected, as the GST domain does not have any relevant biological activity as far as FIP is concerned.

Example 1. Isolation and Purification of Native Fve Protein from Golden Needle Mushroom

Methods and materials

Two kilograms of the fruit bodies of *Flammulina velutipes* are homogenized with 2L ice-cold 5% acetic acid in the presence of 0.05 M 2-mercaptoethanol and 0.3 M sodium chloride. The proteins in the supernatant are precipitated by 95% saturated ammonium sulfate.

The precipitate is re-dissolved and dialyzed against 10 mM Tris-HCl pH 8.5 (buffer A) at 4°C for 48 hours with six to eight changes of dialysis buffer. The protein solution is applied to the Q Sepharose FF column (2.6 × 10 cm, Pharmacia) that has been previously equilibrated with buffer A. The unbound fraction is collected and dialyzed against 10 mM sodium acetate pH 5.0 (buffer B) at 4°C for 48 hours with six to eight changes of dialysis buffer and then further purified by applying to the SP Sepharose FF column (2.6 × 10 cm, Pharmacia) that has been previously equilibrated with buffer B.

The protein is eluted with a gradient of 0-0.5 M NaCl in buffer B. Fractions containing Fve protein are collected and analyzed by a 7.5% Tris-Tricine SDS-PAGE.

Results

High yield of native Fve protein is purified from Flammulina velutipes

The native Fve protein has an apparent molecular weight of 12.7 kDa as determined by SDS-PAGE (Figure 1A). However, it appears to be a homodimer with a molecular weight of 25.5 kDa as determined by Superdex 75 (26 × 60 cm, Pharmacia) gel filtration chromatography (Figure 1B and 1C). The running buffer for gel filtration is 10 mM Tris-HCl pH 7.5, 0.2 M sodium chloride.

Fve protein is the major component in the crude extract from the mushroom fruit bodies. By removing the cap of the mushroom, we managed to reduce the amount of polysaccharides that cause undesirable interference in the process of protein purification.

The yield of native Fve protein is 40 mg from 1 kg wet-weight of starting material.

Example 2. Measurement of gene expression profile at mRNA level after Fve stimulation

Methods and Materials

Two subsets of effector Th cells have been defined on the basis of their distinct cytokine secretion patterns and immunomodulatory effects (Mosmann et al., 1989; Paul and seder, 1994; Abbas et al., 1996). Th1 cells produce inflammatory cytokines, such as IFN-γ, TNF-α, IL-12, IL-15 and IL-18, and enhance cellular immunity mediated by macrophages. In contrast, Th2 cells produce a different group of cytokines, such as IL-4, IL-5, IL-6 and IL-13. The differentiation of precursor T cells into Th1 or Th2 cells has important biologic implication in terms of susceptibility or resistance to a particular disease.

In order to characterize the cytokines expression pattern induced by Fve, human PBMC from healthy donor and splenocytes from 8 week-old BALB/cJ mice are collected and cultured with 20μg of native Fve . The mRNA is extracted at 48 hours using RNeasy Mini mRNA Purification Kit (QIAGEN). First-strand cDNA is then generated from the mRNA template using oligo-dT primers and MMLV reverse transcriptase (Promega).

PCR reactions are performed with Taq polymerase (Promega) with standard conditions and optimized annealing temperatures. The amplified products are analysed by electrophoresis in 1.5% agarose gel containing ethidium bromide (0.5μg /ml) and photographed with UV exposure. Messenger RNA for various cytokines and transcription factors are measured. House keeping genes mRNA for hypoxanthine ribosyl-transferase (HPRT) and cyclophilin are used as internal controls.

Results

Enhanced expression of IFN-γ, TNF-α, IL-1β, IL-2, IRF-1, c-Rel, Bcl-X_L, ICAM-1, and iNOS mRNA

Human PBMC and spleen cells from BALB/cJ mice are cultured with 20μg of Fve and analyzed for cytokine mRNA expression at 48hr. The results indicated that there is an increase in IFN-γ, TNF-α, iNOS mRNA production by spleen cells cultured with Fve protein. Mouse IL-12 remains unchanged. This phenomenon occurred in a dose dependent manner.

Similar results are seen in human PBMC. The mRNA for human cytokines IL-1β, IL-2, IFN-γ and TNF-α; transcription factor IRF-1 and c-Rel; adhesion molecule ICAM-1 and anti-apoptotic protein Bcl-X_L is up regulated after Fve stimulation. Figure 2 and Figure 3 show the patterns of mRNA expression for transcription factors, cytokines and adhesion molecules of the splenocytes and PBMC stimulated by Fve.

Example 3. Generation of Fve Mutants By PCR-Based Mutagenesis

Materials and Methods

A cDNA encoding for the Fve protein is cloned into the BamHI and EcoRI site of pGEX-4T1. This DNA template is used to generate a panel of mutants by recombinant-PCR method (Figure 4). A schematic representation of the strategy used to generate mutants is shown in Figure 5.

As predicted by PHD prediction program, Fve contains one α-helix, six β-strands and two β-turns. Each of these predicted secondary structures is serially deleted by recombinant-PCR method. In addition, we also examined the potential function of the R27, G28, T29 residues, which resembles the cell aggregating RGD motif, located in the N-terminal β-turn of Fve protein by point mutation. Each of the amino acid residues of RGT is substituted by alanine residue.

A partial list of fragments of Fve is shown in **Appendix B.**

Example 4. Production of the Fve-Derived Mutant Proteins

Materials and methods

Eleven deletion mutants and three point mutants of Fve DNA are generated. Each of the polypeptides is expressed in TG1 *E.coli* cells as fusion protein with GST carrier protein and purified by glutathione affinity column. All the mutants could express protein except insoluble mutant D6-18, in which α-helix has been deleted.

Figure 6 shows the panel of the affinity purified mutant proteins on a SDS-PAGE. These purified proteins are used for the cell aggregation, hemagglutination and lymphocytes proliferation assay.

Example 5. Comparison of Hemagglutination Activity of Fve Mutants

Materials and methods

5ml of whole human blood obtained from a healthy volunteer is centrifuged at 2500Xg for 10min. The plasma is removed and 2ml of packed red blood cells are collected from the bottom of the tube.

The red blood cells (RBC) are diluted 5X with 1xPBS buffer and centrifuged at 1200Xg for 10min. RBC pellet is resuspended in 1.5%(v/v) of 1xPBS. 50ul of 2x serial dilutions (from 64μg /ml to 0.25μg /ml) of each Fve mutant protein is added into 50ul of 0.2% gelatin in 1xPBS (pH 7.4) and then mixed with 100ul of 1.5% RBC in each well of the 96-well round bottom microtiter plates. Cells are incubated at room temperature and examined for hemagglutination after 2 hours and over night, respectively (Table 1).

Example 6. Lymphocytes Aggregation Activity of Fve and Its Mutants

Materials and methods

Human peripheral blood mononuclear cells (PBMC) from a healthy donor are isolated and cells are then cultured with 20μg /ml of various Fve mutants in 24-well plates. Cells aggregation is observed by inverted light microscopy after 24 hours (Table 1).

Results

Mutant GST-FveG28A lost the hemagglutination and lymphocytes aggregation activity

Native Fve, GST-Fve (wild type) and two point mutants, GST-FveR27A and GST-FveT29A, show positive aggregation and hemagglutination activity. These properties are not seen in all the deletion mutants and a point mutant GST-FveG28A. PHA and ConA are used as positive controls; GST and Blo t 5 are used as negative controls. These results are summarized in Table 1.

The Arg-Gly-Asp (RGD) tripeptide sequence is the most common molecular recognition site implicated in several immunological reactions. Normally RGD motif is located in the β-turn structure. According to the PHD prediction, residue 19 to 33 is a β-turn structure. Therefore, we propose that glycine residue of RGT (RGD-like motif) tripeptide sequence at position 28 plays an important role on lymphocyte aggregation/adhesion. The potentially interaction between Fve and the proteins of integrin family will be addressed.

	Cell aggregation	Hemagglutination
D19-33	-	-
D34-46	-	-
D47-60	-	-
D61-72	-	-
D73-84	-	-
D85-97	-	-
D98-106	-	-
D107-115	-	-
P55-100	-	-

D61-97	-	-
*R27A	+	+
**G28A	-	-
***T29A	+	+
rGST-Fve	+	+
nFve	+	+
GST	-	-
Blo t 5	-	-
ConA	+	+
PHA	+	+

Table 1. Lymphocytes aggregation and RBC hemagglutination activities of Fve mutants

Example 7. Lymphoproliferation Activity of Fve Mutants

Materials and methods

Splenocytes from Balb/cJ mice and peripheral blood mononuclear cells (PBMC) from a healthy donor are stimulated with 2.5μg /ml, 5μg /ml, 10μg /ml or 20μg /ml respectively of Fve mutant proteins for 24 hours. Then 1 μCi [^{3}H]-thymidine is added to the culture and further incubated for 18 hours. [^{3}H]-thymidine incorporation is measured in triplicates by a β counter (Beckman).

Results

Figure 7 and 8 show the results of the proliferation assay for the panel of proteins tested. Deletion mutants D19-33, D73-84, P55-100, and mutant with single amino acid substitution G28A showed significant reduction in lymphoproliferation activity in mouse splenocytes, whereas, such reduction is less pronounced for the rest of the mutants tested (Figure 7).

Interestingly, some mutants such as D34-46, D47-60 and D61-72, which show negative hemagglutination and cell aggregation, retain similar lypmphoproliferative

activity as the wild type protein. For the result of human PBMC, deletion mutant D61-72 and mutant with single amino acid substitution G28A show more than 50% reduction in lymphoproliferation activity (Figure 8). Taken together the proliferation results from mouse splenocytes and human PBMC demonstrate that glycine at position 28 plays an key role in lymphocyte proliferation.

Example 8. Recombinant GST-Fve (Wild Type) and GST-FveT29A (Mutant) Show Similar Proliferative Activity of $CD3^+$ T Cells as the Native Fve

Materials and methods

Human peripheral blood mononuclear cells (PBMC) from a healthy donor are isolated according to the standard protocol (Coligan et al., 1998). The cells are then cultured with 20μg /ml of recombinant wild type GST-Fve and mutant GST-FveT29A for 5 days. Cells are stained with anti-$CD3^+$ PerCP monoclonal antibody (Becton Dickinson), and analyzed by FACScan flow cytometry (Becton Dickinson).

Results

A histogram shows that 8% and 17% enrichment of T cells are detected after stimulation with recombinant wild type GST-Fve and mutant GST-FveT29A for 5 days (Figure 9). Results showed that both recombinant wild type GST-Fve and mutant GST-FveT29A showed comparable lymphoproliferative activity of T lymphocytes as well as the native Fve protein.

These data suggest that Fve-mediated T cell polarization and enrichment is detectable at day 5.

Example 9. Detection of IFN-γ and TNF-α by Intracellular Cytokine Staining After Stimulation with Recombinant GST-Fve Protein

Methods and Materials

Intracellular cytokine staining is done by modification of a standard method from PharMingen. Briefly, human PBMC are stimulated *in vitro* with 20μg of native Fve protein, GST, recombinant GST-Fve, GST-R27A, GST-G28A, or with GST-T29A. GlogiPlug™ (PharMingen) is added 48hr after the cultures are initiated, cells are collected 14 hr later and then stained for T cells surface marker (CD3) in FACS buffer containing GlogiPlug™. Cells are then treated with Cytofix/Cytoperm (PharMingen) for 30min. Cells are incubated with cytokine antibodies for 30min after washing with Perm Wash buffer (PharMingen). Finally, cells are washed with PBS containing 1% paraformaldehyde and then analyzed by FACSCalibur flow cytometry (BD Biosciences). CellQuest software (BD Biosciences) is used for data analysis.

Results

The results show that native Fve protein is able to stimulate production of IL-2, IFN-γ, TNF-α, but not IL-4 in $CD3^+$ T cells (Figure 10). Similar results are seen for the recombinant wild type GST-Fve and two mutants GST-FveR27A, GST-FveT29A. Strikingly, recombinant mutant GST-FveG28A failed to stimulate the production of such cytokines (Figure 11 and 12).

The percentages of IFN-γ production induced by GST, GST-Fve, GST-FveR27A, GST-FveG28A, GST-FveT29A are 0.8%, 12.3%, 14.3%, 1.8%, 17.6%, respectively. In contrast, the percentages of TNF-α production which induced by GST, GST-Fve, GST-FveR27A, GST-FveG28A, GST-FveT29A are 1.2%, 21.5%, 18.7%, 1.5%, 14.4%, respectively (Table 2). This data provides further evidence that the glycine residue at position 28 of Fve protein plays an important role in the biological function such as aggregation/adhesion, cytokines production, proliferation, and differentiation of lymphocytes. Further examination of the physiological role of RGT sequence in Fve protein by using blocking monoclonal antibodies and peptide inhibition assay are carried

out to confirm this function. The possibility of integrin-mediated T/NK-cell adhesion is also investigated.

In summary, mutants FveR27A and FveT29A show enhanced mitogenic activities as compared to that of wild type Fve. In addition, the solubility of both mutant proteins is significantly increased in comparison with that of wild type Fve. This improved solubility will greatly facilitate the large scale production of such recombinant protein.

Recombinant proteins	Intracellular IFN-γ	Intracellular TNF-α
GST	0.8%	1.2%
GST-FveWT	12.3%	21.5%
GST-FveR27A	14.3%	18.7%
GST-FveG28A	**1.8%**	**1.5%**
GST-FveT29A	17.6%	14.4%

Table 2: The percentage of intracellular cytokines production in $CD3^+$ T lymphocytes during stimulation with three different Fve mutants with single amino acid substitution

Example 10. Applications of Fve in Allergy

The increasing prevalence of atopic diseases such as hayfever or allergic asthma is a major problem in most developing and developed countries. Accumulating evidence indicates that appropriate immunotherapy prevents the onset of new sensitization and the progress of allergic rhinitis to asthma.

The central role of allergen-specific Th2 cells in the regulation of allergic inflammation has been highlighted. Exploration of novel and effective treatment for atopic diseases is active area of allergy research. Induction of allergen-specific T regulatory immune response, suppression of the effects of IL-4, IL-5 and IL-13 cytokines, and redirecting/balancing Th2 immune response in allergy is an attractive and promising approach to pursue (Akbari et al., 2002; Scanga and Le Gros, 2000; Zuany-Amorim et al., 2002).

Our *in vitro* and *in vivo* studies reveal that Fve interacts with T and NK cells.

Fve-activated T cells produce Th1-skewed cytokines in high levels, and suppress Th2 cytokines (IL-4 and IL-13) production. Thus these biological activities of Fve can be exploited to treat Th2-associated diseases such allergic asthma and rhinitis. The use of the immunomodulatory properties of Fve to treat allergic diseases is novel because there are a number of differences between Fve approach and other existing methods such as hexameric motifs, called CpG motifs or DNA immunostimulatory sequences (ISS).

The function of ISS is act as a danger signal to stimulate non-specific innate immune response (Krieg 2000). It is known that ISS is recognized by the toll-like receptor 9 on B cells and $CD123^+$ dendritic cells. It is unexpected that TLR9 is also involved in autoimmunity (Leadbetter et al., 2002; Krieg 2002; Vinuesa and Goodnow, 2002). Upon the detection of CpG motifs or ISS element, B cells are induced to proliferate and secrete immunoglobulin (Ig), and dendritic cells (DCs) secrete a wide array of cytokines, interferons and chemokines that promote T helper type 1 (Th1) cells. Both B and DCs up-regulate costimulatory molecules and have enhanced abilities to induce Th1 cell immune responses. In contrast, Fve is directly target on T and NK cells to involve in the acquire immunity.

Example 11. *In vivo* Study of the Adjuvant Effect of Fve Using a Murine Allergic Asthma Model

Immunotherapy with recombinant allergen in combination with certain immunomodulator enhancing Th1 but suppressing Th2 immune response is a novel approach to achieve higher efficacies in immunotherapy. Since Fve protein is an activator of Th1/Tc1 immune response, it may be used as such an immunomodulator to provide the adjuvant effects to enhance Th1-skewed immunity.

We investigated the adjuvant effects of Fve for allergy immunotherapy with a combination of a recombinant house dust mite major allergen, Der p 2 and Fve using an animal model.

Methods and Materials

A schematic representation of the experimental design is shown in Figure 13.

8 to 10 week old male BALB/cJ mice obtained from the Sembawang Laboratory Animal Center of Singapore are divided into two groups for each experiment. Mice are sensitized by intraperitoneal injection of 10μg of recombinant Der p 2 in aluminum hydroxide at day 0 and day 21. Twenty-eight days after the sensitization, each group of mice is subcutaneously injected with 50μg of Der p 2 and 50μg of Der p 2 plus 40μg of Fve, respectively. A total of six injections are performed at every alternative day over a period of 12 days. Mice are then challenged with the third intraperitoneal injection of 10μg of Der p 2 plus aluminum hydroxide at day 42. Der p 2-specific IgG1 and IgG2a are determined weekly starting at day 14 by ELISA. Since IgG2a is the hallmark of Th1 immunity in mouse, titer of IgG2a is used a measure of Th1 immunity.

Results

Increase allergen-specific IgG2a production in the treatment group with combination of Fve and allergen

As shown in Figure 13, mice that are subcutaneously treated with 50μg of Der p 2 alone produced relatively lower titers of Der p 2-specific IgG2a, whereas mice treated with 50μg of Der p 2 plus 40μg of Fve showed a significant boost of Der p 2-specific IgG2a production (Figure 14).

Upon challenge with intraperitoneal immunization of Der p 2 in alum at day 42, the Der p 2-specific IgG2a in Fve administered mice is further increased at day 49. It is interesting to note that the Fve-specific IgG1 and IgG2a remained low (data not shown). Similar results are observed in similar experiments performed with another house dust mite major allergen, Blo t 5, from *Bromia tropicalis* (data not shown).

Taken together, the data suggested that Fve protein may act as a potent adjuvant/immunomodulator to boost antigen-specific Th1-skewed immune response, therefore it may serves as a useful reagent to improve the efficacies of immunotherapeutic treatment of allergy in humans. The adjuvanticity and immunomodulatory property of Fve protein may be improved by biomolecular engineering.

While not wishing to be bound by theory, it is postulated that this molecule may activate NK cells and $CD8^+$ T cells and thus result in production of IFN-γ. These may induce a strong cellular-mediated immune response and promote isotype switching to specific IgG2a predominantly.

Example 12. Assessment of Erythema Flare and Wheal Diameter Formation Induced by Skin Prick Tests in Human Allergic Subject

Materials and methods

The skin prick test is a convenient diagnostic method test for allergy in the clinics. The aim of this study is to evaluate the suppression effect of Fve protein to allergen hypersensitivity. As an *in vivo* topical challenge method, the skin prick test is administered to a human subject with history of sensitization to house dust mite *Dermatophagoides pteronyssinus*.

25μg /ml of purified recombinant Der p 2 allergen mixed with same concentration of native Fve protein or Der p 2 allergen alone, is applied to the skin of left and right hand of human subject for 10 minutes. Histamine is used as a positive control. The size of the wheel and erythematic flare diameter is measured manually.

Results

Fve reducse wheal and erythematic flare formation on Der p 2 skin prick test-positive human subject

The formation of wheal and erythematic flare could be detected in the challenged site of histamine, Der p 2, and Der p 2 combined with Fve. The diameter of the wheals in

both left and right hand induced by Der p 2 is 22mm and 24mm, respectively. Interestingly, the mixture of Der p 2 and Fve reduces the wheal's diameter in both hands to 15mm and 18mm, respectively (Figure 15A). A similar reduction is also seen in the size of erythematic flare (Figure 15B, Table 3A and 3B).

The data indicats that there is a suppression of allergic reaction mediated by immunomodulatory effects of Fve protein. The results provide additional evidence that Fve could be used as an adjuvant for allergens immunotherapy.

Besides indoor allergens, outdoor allergens are also important triggering factors that lead to allergic diseases. Hay fever and allergic asthma triggered by grass pollen allergens affect approximately 20% of the population in cool temperate climates. Worldwide more than 200 million individuals are allergic to group 1 grass pollen allergens, and over 100 million individuals exhibit IgE-mediated allergic reactions against Phl p 2, a major allergen from timothy grass (*Phleum pratense*) pollen.

Therefore, we propose that recombinant Fve as well as the native Fve may also be applied in the treatment of other allergies that induced by tree pollen allergen (Bet v 1 and Bet v 2 from birch), grass pollen allergen (Phl p 1 and Phl p 2 from timothy grass), weed pollen allergen (antigen E from ragweed), major feline antigen (Fel d 1), major canine allergen (Der f 15), etc. Other allergens will be known to the person skilled in the art.

Another useful application of Fve protein in allergy is to conjugate or co-deliver with allergenic crude extracts such as mite extracts, pollen extracts, cat and dog extracts, cockroach extracts, fungal and mold extracts for desensitization by immunotherapy.

	Wheal Diameter (mm)	
	Left hand	Right hand
Saline (negative control)	0	0
Histamine	7	5
Der p 2	22	24
Der p 2 + Fve (1:1 w/w)	15	18

Table 3A: Wheal formation on skin after challenged with Der p 2

	Erythematic Flare Diameter (mm)	
	Left hand	Right hand
Saline (negative control)	0	0
Histamine	30x25	35x30
Der p 2	55x40	50x43
Der p 2 + Fve (1:1 w/w)	45x35	45x35

Table 3B: Erythematic flare formation on skin after challenge with Der p 2

FVE ADJUVANTED ALLERGEN VACCINES

Example 13. Fusion Proteins of Fve and Allergen

Materials and methods

Treatment of recombinant allergen or vaccination with naked DNA encoding a specific allergen has been shown previously to elevate allergen-specific Th1 immune response against Th2 immune reaction (Maecker et al., 2001). To enhance the effectiveness of immunotherapy or DNA vaccine therapy, we generated several fusion proteins consisting of the complete Fve molecule and the mature form of Blo t 5 or Der p 2 allergen. Figure 16 shows the construction of seven fusion proteins of Fve and major house dust mite allergen from *Dermatophagoides ptenyssinus* and *Blomia tropicalis*

The fused cDNAs are successfully expressed in E coli (Figure 17) and the biological properties of the recombinant proteins are examined.

Results

The morphology of lymphocyte culture upon stimulation with three recombinant fusion proteins is photographed with inverted microscope (Figure 18A-C). Each of Bt5-Fve, Bt5-FveR27, GST-Dp2-FveR27 are able to increase the number of human PBMC (Figure 19A and 19B), to stimulate the proliferation of human lymphocytes (Figure 20), to polarize human $CD8^+$ T cells (Figure 21), and to increase the production of IFN-γ (Th1 response) and IL-10 (Tr response) (Figure 22).

A well-balanced vaccine that induces both Th1 and Tr immune response may be the most valuable and desirable. The Th1 response may very efficiently inhibit the development of Th2 cells via IFN-γ, leading to a life-long protective Th1 memory immune response. Allergen specific Tr cells may in turn dampen the anti-allergic Th1 immune response, ensuring a well-balanced protective but nonpathological Th1 response. Allergen-Fve fusion proteins meet these criteria since they induce cytokine IL-10.

Thus, combining Fve protein with allergen in the form of a fusion protein may be used effectively to induce antigen-specific adjuvant effect that augment the Th1 and Tr responses, which in turn down-regulate the Th2 allergic responses.

To test the antigenecity of a Blo t 5-Fve fusion protein, competitive inhibition ELISA is performed using varying concentrations of proteins (GST, GST-Blo t5, GST-Fve, GST-Blo t5-Fve, GST-Fve-Blo t5, Blo t5-Fve). The results show that fusion protein Blo t 5-Fve, un-cleaved GST-Blo t5-Fve and GST-Fve-Blo t5 have lower IgE binding affinity compared to Blo t5 alone and un-cleaved GST-Blo t5 (Figure 23). The fusion protein Blo t5-Fve inhibited IgE binding to a maximum of 70% whereas Blo t5 is able to inhibit the binding of antibody to GST-Bt5 to 100% at inhibitor concentration of 10 μg/ml. Control GST and GST-Fve are not able to inhibit the binding of IgE to GST-Blo t5 (background levels). In summary, there is a reduction in the IgE binding affinity of Blo t5 when it is in the fusion forms of Blo t5-Fve, GST-Blo t5-Fve and GST-Fve-Blo t5 indicating that the antigenicity of Blo t5 with Fve in fusion forms is lowered.

Example 14. Allergen Conjugated to Fve

Beside the use of gene fusions to produce fusion proteins, protein-protein conjugation also provides a convenient and alternative choice to develop allergen vaccine.

To date, allergen conjugated adjuvants which have been reported include crystalline bacteria cell surface layer (S-layers) (Jahn-Schmid et al., 1996), CpG

oligodeoxynucleotides (CpG motifs) (Shirota et al., 2000), cholera toxin B subunit (CTB) (Rask et al., 2000), and Brucella abortus (Scharf et al., 2001).

Here we disclose Fve protein which is isolated from edible mushroom can also be an ideal adjuvant coupling to allergen vaccine. Poly-lactic acid (PLA) and polyethylene glycol (PEG) are two materials which may be used to couple Fve and house dust mite allergen (Der p 2 or Blo t 5), although other materials will be evident to the skilled reader.

Particular coss-linking reagents which may be used to conjugate an allergen and immumodulator, such as Fve, include N,N'-dicyclohexylcarbodiimide (DCC), N-succinimidyl-S-acetyl-thioacetate (SATA), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), ortho-phenylenedimaleimide (o-PDM), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC). A chemical conjugation protocol which may be used is that provided in the Protein-Protein Crosslinking Kit (P6305) from Molecular Probes, Eugene, USA. Protocols for conjugation using SPDP are disclosed in Clinical Experimental Allergy 30: 1024-1032, 2000 and European Journal of Immunology 28: 424-432, 1998.

For example, native Fve or recombinant Fve from *E coli* is conjugated with N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP, Molecular Probes) as a bifunctional coupling reagent. The resulting Allergen-Fve conjugates are purified by gel filtration and characterized for their allergenicity and adjuvanicity by *in vitro* and *in vivo* assays.

Example 15. Human Cytokine Assay in Purified $CD4^+$ and $CD8^+$ T Cell Subsets

Materials and Methods

To elucidate and identify subsets of human T lymphocytes responding to Fve stimulation, purified $CD4^+$ T cells and $CD8^+$ T cells from four human tonsillectomy patients (subject 1, 6 yrs-old Chinese; subject 2, 16 yrs-old Indian; subject 3, 17 yrs-old Malay; subject 4, 27 yrs-old Malay) are stimulated with 20μg of Fve after AutoMACS

seperation. AutoMACS is an automated magnetic cell sorter from Miltenyi-Biotec, Germany. The differential cytokine production profiles of these subsets are determined by intracellular cytokines staining after 48 hours in vitro culture.

Results

Fve Triggers Th1/Tc1 Cytokine Production in Human T Cells

The human cytokines induction studies show that Fve stimulates the production of IL-2, IFN-γ, TNF-α, whereas IL-4 and IL-10 are nearly undetectable. In addition, purified $CD4^+$ T cells produce higher levels of TNF-α than purified $CD8^+$ T cells ($CD4^+$ vs $CD8^+$: 11.4% vs 2.5%), whereas purified $CD8^+$ T cells produce higher levels of IFN-γ than purified $CD4^+$ T cells ($CD4^+$ vs $CD8^+$: 3.6% vs 8.5%) upon Fve stimulation (Table 4). Therefore, the enrichment of $CD8^+$ T cells seems to derive from a protein-cell direct interaction. Taken together, this data supported that Fve could trigger Th1/TC1 cytokines production in human T lymphocytes.

Intracellular Cytokines Scretion	Purified $CD8^+$ T cells from human tonsil		Purified $CD4^+$ T cells from human tonsil	
	None	Fve	None	Fve
IL-2	0.1%	0.6%	0.2%	**6.8%**
IL-4	0.1%	0.3%	0.1%	0.9%
IL-10	0.6%	0.5%	2.3%	0.9%
IFN-γ	0.1%	**8.5%**	0.6%	**3.6%**
TNF-α	0.2%	**2.5%**	0.4%	**11.4%**

Table 4. Cytokines profile of purified human T cells subsets

Example 16. Lymphocyte Aggregation Activity of Fve

Materials and Methods

Human $CD4^+$ and $CD8^+$ T cells subset are purified from AutoMACS (an automated magnetic cell sorter from Miltenyi-Biotec, Germany). The morphology of the cells is observed by light microscope at day 3.

Six human cell lines are also used for the cell aggregation study. Promyelocytic HL-60 cells, Jurkat-T cells, monocytic leukemia U937 cells, myeloid leukemia K562 cells, Raji B cells, natural killer NK-92 cells are cultured with native Fve protein with 2.5μg/ml, 5μg/ml, 10μg/ml, 20μg /ml and 40μg/ml, respectively. Cells aggregation is observed by inverted light microscopy after 24 hours.

Results

Fve induced aggregation of human $CD4^+$ and $CD8^+$ T cells subsets, HL-60, Jurkat-T cells, and NK-92 Cells

Human $CD4^+$ and $CD8^+$ T cells subset are purified from the tonsil of human subject. The aggregation of $CD4^+$ and $CD8^+$ T cells upon stimulation with 20μg of Fve protein is observed by confocal microscope at day 3 (photographed data not shown).

From the human cell line study, we found that Fve could induce HL-60 aggregation at low concentration of 2.5μg /ml. Jurkats-T cells and NK-92 also induced aggregation by Fve at concentration of 10μg /ml and 20μg /ml, respectively, where as U937, K562 and Raji didn't induce cell aggregation (Table 5). From the result, it seems that the level of cell aggregation correlates with the level of certain surface protein(s) expression in different cell lines. Promyelocytic cell line HL-60 seems to be an idea cell line to identify Fve receptor.

Human Cell Lines	Fve				
	2.5μg /ml	5μg /ml	10μg /ml	20μg /ml	40μg /ml
HL-60	+	+	+	+	+
Jurkat T	+/-	+/-	+	+	+
U937	-	-	-	-	+/-
K562	-	-	-	-	+/-
Raji	-	-	-	-	-
NK-92	-	-	+/-	+	+

Table 5. Cell aggregation activity of human cell lines

Example 17. *In vitro* Polarization of Human NK cells and CD8 $^+$ T Cells

Materials and Methods

Human peripheral blood mononuclear cells (PBMC) from a healthy donor are isolated as standard protocol (Coligan et al., 1998). The cells are then cultured in 24-well plates with native Fve (5μg /ml or 25μg /ml). At days 5 and 10, cell culture are stained with anti-CD4$^+$ FITC, anti-CD8$^+$ PE, anti-CD16$^+$ PE plus anti-CD56$^+$ PE monoclonal antibodies (Becton Dickinson), and analyzed by FACScan flow cytometry (Becton Dickinson).

Results

Sequential polarization of cells by Fve, NK cells and NKT cells are proportionally increased at day 5 whereas CD8$^+$ T cells are increased at day 10

The results show a 10% increase of CD16$^+$ and CD56$^+$ double positive cells (Natural Killer cells) after stimulation with Fve protein for 5 days (Figure 24). In addition, CD8$^+$ T cells but not CD4$^+$ cells are increased up to 35% after culturing for 10 days (Figure 25). This result showed that native Fve protein could stimulate both natural killer

cells and $CD8^+$ T cells and the stimulation of these cells occurred sequentially with polarization of NK cells and $CD8^+$ T cells peaked at day 5 and day 10, respectively.

The data also showed that cell culture consisted of 10% of $CD3^+CD16^+CD56^+$ NKT cells after stimulation with 25μg /ml of native Fve protein (Figure 24E). This subset of cytotoxic NKT cells has a unique feature in that they mediate non-MHC-restricted cytotoxicity (Lanier et al., 1986).

Example 18. Up- Regulation of a Novel Subset of $CD8^+$ T Cells ($CD3^+$ $CD8^+$ $CD18^{+\,bright}$)

Materials and Methods

Repeated subcutaneous injection of IL-12 in patients with cancer resulted in the selective expansion of a unique subset of peripheral blood $CD8^+$ T cells. This subset expressed high levels of $CD18^+$ and up- regulated IL-12 receptor expression after IL-12 treatment in vivo. They appeared morphologically as large granular lymphocytes, increased high IFN-γ production and enhanced non-MHC-restricted cytolytic activity. Thus, these T cells may play an important role in innate as well as acquired immunity to tumors and infectious pathogens.

To determine whether $CD3^+$ $CD8^+$ $CD18^{+\,bright}$ T cells can be enriched by native Fve protein, human peripheral blood mononuclear cells (PBMC) from a healthy donor are isolated and cultured with 20μg /ml of native Fve protein. Cell culture are stained with anti-CD18 FITC, anti-CD8 PE, anti-CD3 PerCP monoclonal antiboodies (Becton Dickinson) at day 5, and then analyzed by FACSCalibur flow cytometry (Becton Dickinson).

Results

Result showed that $CD3^+CD18^{+bright}$ T cells are increased from 8% to 31% of total cell population (Figure 26), and $CD3^+CD8^{+bright}CD18^{+bright}$ T cells are increased nearly three times, from 3.5% to 9% of the total cell population (Figure 27) after stimulation with

20μg /ml of native Fve protein. Furthermore, some $CD18^{+}CD8^{-}$ cells started to differentiate into $CD18^{+}CD8^{+dim}$ cells after stimulated with native Fve protein (Figure 27B). This data suggested that Fve protein from the golden needle mushroom has a potential ability to stimulate cellular immune responses directed against malignancies in human.

Example 19. *In vivo* Lymphocyte Proliferation Assays

Materials and Methods

Since Fve protein can activate human NK cells and $CD8^{+}$ T cells *in vitro*, we sought to determine whether Fve would enhance activation of these cells *in vivo*. Mouse provides a good model system for such a study.

A group of three C57BL/6J mice are subcutaneously injected with 10μg, 50μg or 250μg Fve protein consecutively for three days, respectively. Another three BALB/cJ mice are treated with 125μg of Fve protein each for seven days by subcutaneous injection. For continuous BrdU labeling, mice are given 0.5mg/ml BrdU (Sigma) in the drinking water, which is changed every 3 days and then each mouse received one intraperitoneal injection of 1mg of BrdU in PBS at 6 hours before being sacrificed. PBMC, lymph node and spleen are isolated and resuspended in 200ul of washing buffer (1xPBS containing 1% bovine calf serum), then stained with anti $CD4^{+}$-FITC, anti $CD8^{+}$-PE, anti $CD19^{+}$-PE or anti PanNK-PE monoclonal antibody (Pharmingen) for 30 minutes on ice. After two washings with washing buffer, the samples are fixed with FACS Permeabilizing Solution (Becton Dickinson) for 16 hours. After that samples are treated with 50U DNase I (Sigma) for 1hr at room temperature. The cells are washed and stained with anti BrdU-FITC mAb (Becton Dickinson) in PBS for 30 minutes. 1-5 x 10^{5} viable (forward and side scatter gated) PBMC, lymphocytes in lymph nodes or splenocytes per sample are analyzed with FACScan (Becton Dickinson) and data are processed using the CellQuest software (Becton Dickinson).

Results

Fve induced NK cells and CD8 $^{+}$ T cells proliferation in vivo

FACScan analysis data showed that Fve could induce increased proliferation of NK cells and $CD8^{+}$ T cells in a dose-dependent manner in C57BL/6J mice (Figure 28 and Figure 29). In contrast, $CD4^{+}$ T cells and $CD19^{+}$ B cells showed no significant increase (Figure 30 and Figure 31). Similar $CD8^{+}$ T cell polarization is also seen in lymph nodes of C57BL/6J mice (Figure 32) and so the peripheral blood mononuclear cells (PBMC) of Balb/cJ mice that are subcutaneous injected for seven consecutive days with 125μg of Fve protein. The $CD8^{+}$ versus $CD4^{+}$ T cells ratio is significantly increased in each of the Fve-treated BALB/cJ mouse as compared to the naïve control (Figure 33). Data from the experiment are presented in Table 6 below.

Naïve Balb/cJ mouse	PBMC		
	$CD4^{+}$ T cells	$CD8^{+}$ T cells	$CD8^{+}/CD4^{+}$ ratio
#1 None	40.3 %	15.7 %	0.389
#2 125μg nFve	40.2 %	**26.2 %**	**0.651**
#3 125μg nFve	40.7 %	**21.8 %**	**0.535**
#4 125μg nFve	33.3 %	**19.6 %**	**0.588**

Table 6. Data showing results of Figure 33.

In summary, for NK cells in spleen, 10μg Fve caused one fold increase proliferation. The proliferation increased to 5-6 fold when 50μg and 250μg of Fve protein is added. Similar finding is observed in CD8 positive T cells in spleen and lymph nodes. 250μg Fve protein caused 2-3 fold increase proliferation as compared to non-treated mouse. By contrast, Fve failed to stimulate CD4 positive T cells and has very mild stimulation to CD19 B cells (Table 7). Similar phenomenon is also seen in the peripheral blood mononuclear cells. The proportional of CD8 T cells increased up to 6-10% after 125μg of Fve protein are subcutaneous injected to Balb/cJ mice for seven days (Table 8).

These *in vivo* data are in concordance with those derived from *in vitro* studies, which clearly indicate that Fve induces selective polarization of NK cells and $CD8^+$ T cells. Furthermore, these immunostimulatory effects of Fve are independent of the genetic background of mouse strains. Thus, Fve appears to be a potent immunostimulator for cellular mediated immune response. Purified NK cells and $CD8^+$ T cells will be used for future studies to examine the molecular and cellular basis for the polarization of cell subsets.

Naïve C57BL/6J mouse	Spleen				Lymph nodes
	BrdU incorporated NK cells	BrdU incorporated $CD4^+$ T cells	BrdU incorporated $CD8^+$ T cells	BrdU incorporated $CD19^+$ B cells	BrdU incorporated $CD8^+$ T cells
#1 None	0.63%	3.49 %	2.22 %	3.48 %	5.83 %
#2 10μg Fve	1.20 %	3.32 %	2.81 %	3.43 %	5.72 %
#3 50μg Fve	3.53 %	3.47 %	3.34 %	4.11 %	9.19 %
#4 250μg Fve	**4.00 %**	2.55 %	**7.31 %**	4.55 %	**12.05 %**

Table 7. *In vivo* stimulation of C57BL/6J mouse lymphocytes

Naïve Balb/cJ mouse	PBMC		
	$CD4^+$ T cells	$CD8^+$ T cells	$CD8^+/CD4^+$ ratio
#1 None	40.3 %	15.7 %	0.389
#2 125μg Fve	40.2 %	26.2 %	**0.651**
#3 125μg Fve	40.7 %	21.8 %	**0.535**
#4 125μg Fve	33.3 %	19.6 %	**0.588**

Table 8. *In vivo* stimulation of Balb/cJ mouse lymphocytes

Example 20. *In vivo* Evaluation of the Potential Use of Fve for Immunotherapy of Solid Tumors

There are several approaches to treat cancer such as surgery; radiation therapy; given tumor cell arrested drugs; induced apoptosis in cancerous cells; inhibited angiogenesis; elevated tumor recognition and specific killing ability of immune system to eliminate cancerous cells.

Previous data have indicated that Fve protein stimulate enhanced production of various cytokines, particularly IFN-γ, TNF-α and IL-2; induced polarization of natural killer cells and $CD8^+$ T lymphocytes; and triggered a Th1/Tc1-like cellular-mediated immune response. Each of these biological properties may contribute to suppression of tumor growth and to prevent the risk of cancer recurrence by inducing various forms of nonspecific or even specific immunity after surgery.

Malignant melanoma is a very common cancer in the western world. A subset of patient with metastatic melanoma can be successfully treated by the administration of recombinant IL-2, sometimes given together with autologous melanoma-reactive lymphocytes that have been expanded ex vivo. Since melanocyte differentiation antigens, including MART-1/Melan-A, gp100, tyrosinase, TRP-1, and TRP-2, and cancer-testis antigens, including MAGE-3, BAGE, GAGE, NY-ESO-1, are recognized by human T lymphocytes, therefore they become the attractive targets for melanoma vaccines. However, from an immunological point of view, these melanocytes differentiation antigens and cancer-testis antigens are "self" antigens. It may induce central or peripheral tolerance, and thus potentially hampering the induction of powerful anti-melanoma immune responses. Therefore, induction of a strong tumor specific immunity with an immunopotentiator or novel adjuvant could be a useful treatment strategy to overcome immune ignorance and tolerance.

In order to investigate the anti-tumor effect of Fve, C57BL/6J mice are subcutaneously inoculated either with T cell lymphoma EL4 or melanoma B16-F1, the

later is a well established and widely used tumor model for which treatment is notoriously difficult. The tumor growth and survival rate of mice are monitored.

Materials and Methods

Construction of pCIneo-fve and pDisplay-fve recombinant plasmid DNA

The pCIneo vector is designed for high level and constitutive expression of cloned DNA inserts in mammalian cells (Figure 34A). Fve DNA is amplified from pGEX-fve and subcloned into the Xho I and EcoR I restriction enzyme cutting sites of pCIneo vector. The pCIneo-fve is used for priming the immune response by intramuscular injection.

The pDisplay vector is a mammalian expression vector that is designed to target and to display recombinant proteins to the surface of mammalian cells (Figure 34B). Fusion DNA of Fve and murine Ig kappa chain V-J2-C signal peptide without hemagglutinin A epitope is generated by recombinant PCR and subcloned into the EcoR I and Pst I restriction enzyme cutting sites of pDisplay vector. The Fve protein expressed from the pDisplay-fve acts as triggering signal for immune system and recruiting T lymphocytes to recognize tumor cells.

Transfection of B16-F1 cells with pDisplay-fve

The murine melanoma cells B16-F1 is purchased from ATCC, USA. Tumor cells are grown in DMEM supplemented with 10% FBS in 5% CO_2. Cells in the exponential growth phase within four passages are used in this investigation. To obtain stable transfectants, endotoxin free plasmid pDisplay-fve is mixed with polyfect transfection reagent (QIAGEN, Germany) and transfected into B16-F1 cells. Colonies resistant to G418 (Geneticin, GIBCO BRL) at 1000μg /ml for 25-30 days are isolated and designated as B16-Fve. The control B16-F1 cells which are transfected with pDisplay vector alone are designated as B16-vec.

EL4 protection assay

Six to eight weeks old C57BL/6J mice are inoculated with $8x10^6$ EL4 cells. Tumor formation is observed at day 3. 100μg of pCIneo-fve recombinant plasmid DNA is intramuscularly injected into the tibialis muscle at day 0 and day 7. 20μg of Fve protein is given by subcutaneous injection surrounding the tumor site at day 5, 7, 9, 11, 13, 15, and 18, respectively. The diameters of tumors are measured with a caliper and tumor volume is calculated by long diameter time short diameter. Finally the survival rate is recorded.

DNA vaccination and B16-F1 tumor protection experiments

Endotoxin free pCIneo and pCIneo-fve are purified from the QIAGEN plasmid DNA extraction and purification kits. 100μg of pCIneo-fve is intramuscularly injected into the tibialis muscle of C57BL/6 mice at day -30 and day -1. Muscles are pulsed with Electro Square Porator ECM830 (BTX, Genetronics, USA) equipped with a two needle array electrode after DNA injection. Mice are inoculated with $5x10^5$ B16-F1 cells. Small tumor nodule developed at day 3. 50μg of Fve protein is given by subcutaneous injection surrounding the tumor site at day 4, 7, 9, and 12, respectively.

Experimental lung metastasis

B16-F1 cells are trypsinized from monolayer cultures, counted and spun down at 1,200 rpm for 5 min and resuspended with DMEM. Five C57BL/6 syngenic 6-week-old female mice are intravenously injected with $2x10^4$ of B16-F1 melanoma cells in a final volume of 120 μl. About 4 weeks after injection, tumor nodules are established in lung. Mice are kept until they died to assess survival.

Example 21. Prolonged Survival Rate of Tumor-Inoculated Mice Receiving with Fve Gene and Protein

Our results show that tumor established mice that received pCIneo-fve DNA and Fve protein had shown a reduction of T cell lymphoma growth rate (Figure 35) and an

extension of survival time (Figure 36). Similar results are also seen in melanoma B16-F1 inoculated C57BL/6J mice (Figure 37).

These data indicate that Fve induces some protection against the solid EL4 tumor and B16-F1 melanoma, suggesting that Fve could be a potential candidate molecule for the development of the immunotherapeutic reagents for treatment of some cancers. The results also show that DNA vaccine-mediated treatment using the gene of Fve can be further exploited for effective cancer treatment. Nowadays, DNA vaccination is being explored as a potential strategy for combating cancer. However, tumor antigens are often weak and the immune system of patients may be compromised. Like the concept of allergen-Fve fusion protein, fusion of Fve to specific tumor antigen may an effective way to activate protective anti-tumor immune response. Genetic immunization with chemeric gene encoding Fve and tumor antigen may augment and direct immune attack on a range of target tumor antigens.

Example 22. Life Span in Solid Tumor Model is Extended in Fve Transfectant

In previous study, we have proved that using Fve plasmid DNA primed in muscle and Fve protein boosted in tumor region could initiate anti-tumor immune response and thus prolong the survival time of tumor-inoculated mice. Instead of injection Fve surrounding the tumor, we specifically targeted Fve gene into tumor cells and tried to create an inducible-antigenic tumor for cancer therapy. This genetically modified tumor cells may provide signals for antigen presenting cells and both helper and cytotoxic T cells.

To determine whether introduction of the Fve gene into malignant cells would result in enhanced tumor recognition ability via Fve display and lead to extended survival rate in solid tumor experiment. Recombinant plasmid DNA pDisplay-fve is transfected into wild type B16-F1 mouse melanoma and then G418 resistant colonies are selected. Five female of C57BL/6J mice are inoculated with $5x10^4$ of B16-Fve transfectant. The antigenicity of B16-vec and B16-Fve transfectants are compared through the life span of two groups of tumor-inoculated mice.

Result demonstrated that artificially expressed Fve on the surface of B16-F1 mouse melanoma extended survival rate as compared to B16-vec inoculated mice (Figure 38). We propose that the characteristics of highly antigenecity and lymphocytes mitogenecity of Fve may elevate immune function to fight against tumor when it displayed on the surface of melanoma. Therefore, Fve may use as immune response activator and enhancer especially for those poorly recognized and low immunogenic tumor, which escaped from cancer surveillance and immune clearance by altering immune recognition and modulating cytotoxic response.

Example 23. Fve **DNA Vaccination Retards Tumor Progression**

Cancer vaccines are designed to prevent and treat cancer. In general, research has shown that the most effective anti-tumor immune responses are achieved by stimulating T cells, which can recognize and kill tumor cells directly. Most current cancer vaccines try to activate T cells directly, try to enlist APCs to activate T cells, or both. Some novel ways in which researchers are attempting to better activate T cells are: (1) Altering tumor cells so molecules that are normally only express on APCs are now express on the tumor cell. These molecules are capable of giving T cells a stronger activating signal than the original tumor cells. (2) Testing more cytokines and adjuvants to determine which are best candidates for recruiting APCs to areas where they are needed. (3) Using dendritic cells and other APCs fused with tumor cells as the cancer vaccines. These cells go into the body carrying tumor antigen and ready to activate T cells. Early cancer vaccine clinical trials involved mainly patients with melanoma. Currently, cancer vaccines are also being tested in the treatment of many other types of cancer, including prostate cancer, breast cancer, colon cancer, and lymphoma.

Here, we accessed tumor immunity that stimulated by recombinant Fve DNA vaccination alone and the combination of Fve DNA vaccination and Fve-transduced tumor cells. C57BL/6J mice are separated into three groups and each group consisted of ten mice. Mice are inoculated either with $5x10^4$ of B16-Fve or B16-vec tumor transfectants in the dorsal back. Tumor formation is observed at day 5-7. 100μg of pCIneo-fve plasmid DNA

is intramuscularly injected at the right and left tribilis muscle of C57BL/6J at day -77, day -35 and day -21. Mice are subcutaneously injected with $5x10^4$ of B16-Fve transfectant and B16-vec transfectant at day 0, respectively. 100μg of pCIneo plasmid DNA is administered following similar experimental procedure and mice are subcutaneously injected with $5x10^4$ of B16-vec transfectant as negative control. The fatal rate of mice are recorded and data are presented as survival curves.

Result showed that Fve DNA vaccination contained certain degree of tumor protection (Green line in Figure 39) as compared with vector DNA vaccination (Blue line in Figure 39). In addition, the combination of Fve DNA vaccination and B16-Fve transfectant exerted a stronger tumor protection effect (Red line in Figure 39). Based on the result, we propose Fve is a novel protein to activate T cells directly. This protein is capable of giving T cells a strong activating signal when it is displayed on the surface of poorly immunogenic tumor cells. Therefore, the phenomenon of extended survival rate is observed in the experimental tumor-inoculated mice.

In future, the adjuvant effect of fusion proteins between Fve and tumor antigens to enhance tumor immunity will be determined. In particular, DNA fusion vaccine strategy, whereby target tumor antigen is genetically linked to immunostimulatory molecules such as Fve, is currently being explored. The introduction of fusion gene encoding tumor-associated antigen with Fve into antigen-presenting cells hold considerable promise for the treatment of patients with cancer. The ease of DNA manipulation has allowed incorporation of a wide variety of molecules able to promote antigen uptake, processing and presentation by professional antigen-presenting cells, to provide critical $CD4^+$ T-cell help and to activate more effective immune effector pathways (Zhu and Stevenson 2002). The concept of DNA fusion vaccine strategy is particularly important for cancer vaccines to increase their immunogenicity and to overcome tolerance.

Example 24. Fve Extends the Survival Rate of Lung Metastatic Mice

$2x10^4$ of B16-F1 melanoma cells is delivered to C57BL/6J via tail vein injection. The effect of combination of distill water and DNA vector pCIneo versus Fve protein and plasmid DNA pCIneo-fve administration on survival after the establishment of lung metastasis is determined. Survival extended in both metastatic experimental groups undergoing Fve protein orally primed and DNA intramuscularly boosted strategy.

Two groups of five C57BL/6J mice are given with 10mg/ml of Fve protein in the drinking water at days -35, -28 and -21, and each water providing is maintained consecutively for one week. Mice are intravenously injected with $2x10^4$ of B16-F1 (wild type) melanoma cells at day 0. One week after, mice are intramuscularly injected with 100μg of pCIneo-fve plasmid DNA into the right and left tribilis muscle, respectively. The mixture of $5x10^4$ of B16-Fve cells lysate plus 10μg of Fve protein (Red line in Figure 40) or 10μg of Fve protein alone (Green line in Figure 40) are subcutaneously injected into mice at the following three weeks. Negative control group of mice received same amount of 1xPBS in the drinking water, intravenously injected with $2x10^4$ of B16-F1 melanoma cells, followed by intramuscularly injected with plasmid DNA vector pCIneo, and finally subcutaneously injected with B16-vec cells lysate plus 1xPBS (Blue line in Figure 40).

Results showed that the strategy of orally primed with Fve protein before tumor introduced into the lung and intramuscularly boosted the immune response with the plasmid DNA pCIneo-fve after tumor established in lung could extend the survival rate of mice as compared with the control group (Figure 40). This data provided another evidence suggesting Fve could augment anti-tumor immune response against developing or metastatic tumor cells. The inhibition of B16-F1 melanoma experimental lung metastasis by Fve may go through induction of IFN-γ, TNF-α and activation of anti-tumor host mechanisms. IFN-$\gamma^{-/-}$ and TNF-$\alpha^{-/-}$ gene knockout mice and in vivo depletions of $CD4^+$, $CD8^+$, or $NK1.1^+$ cells may provide supportive evidence to this phenomenon.

Example 25. Global Gene Expression Profiling of Human T Cells and NK Cells After Activation with Fve

The invention of microarray technology allows the simultaneous monitoring of the transcriptional behavior of thousands of genes. This technology has been repeatedly shown to be useful in the analysis of the response of a variety of cellular systems to stimuli, in the classification of human cancer, and in the analysis of animal models of human disease (Churchill 2002; Slonim 2002; van Berkum and Holstege, 2001). To characterize the transcriptional profile of Fve, we analyzed gene expression patterns in T and NK cells from either healthy donor or human cell lines stimulation with Fve by using oligonucleotide microarrays and compared them with gene expression patterns in non-stimulation cells. In future, protein microarray assays would also be used to study protein–protein interactions on a genome-wide scale (Templin et al., 2002; Zhu et al., 2001).

Materials and Methods

Cells collection and total RNA purification

Peripheral blood mononuclear cells (PBMC) are collected from healthy donors. CD8-positive T lymphocytes and natural killer cells isolation are performed by immunomagnetic bead selection with monoclonal mouse anti-human CD8 antibodies and monoclonal mouse anti-human CD56 antibodies using the AutoMACS automated separation system (Miltenyi-Biotec, Germany). CD8-positive T cells and CD56-positive natural killer cells purity of more than 94% and 88% homogeneity are confirmed by two-color flow cytometry using $CD3^+/CD8^+$ and $CD56^+$ criteria (Becton Dickinson, USA). Human T cell lines (Jurkat T cell, MOLT-4) and NK cell line (NK-92) are grown as recommended (ATCC, USA). Cells are stimulated with Fve and total RNA is isolated with RNeasy Mini Kit (Qiagen, Germany) after 2 and 48 hours, respectively.

Preparation of labeled complementary RNA and hybridization to high-density microarray

Double-stranded complementary DNA (cDNA) and biotinylated complementary RNA (cRNA) are synthesized from total RNA and hybridized to human GeneChip

microarray (Affymetrix, USA), which are washed and scanned according to procedures developed by the manufacturer. The arrays are scanned using laser scanner and visualized using Affymetrix 3.3 software (Affymetrix).

GeneChip data analysis

Differentially expressed genes are analysed by functional assays

Example 26. X-Ray Crystallographic Study of Fve: Materials and Methods

The three dimensional structural of Fve provides a good basis for the understanding of protein functions, immunomodulations and therapeutic applications in allergy and other diseases. We have crystallized the well-diffracting crystals of Fve and show that it diffracts to 1.4 Å resolution when exposed to synchrotron radiation.

This and the follwing Examples describe a 1.6 A° x-ray structure of Fve, determined by Single Anomalous Diffraction (SAD) using the anomalous signal of bromide ions present in the crystal for phasing. Fve represents a novel structure, wherein each monomer consists of an N-terminal α-helix followed by an immunoglobulin fold (beta-sandwich). The structure strongly suggests that dimerization, critical for the activity of FIP proteins, occurs by 3-D domain swapping of these helices and is stabilized predominantly by strong hydrophobic interactions between them.

Crystallization

Fve protein is obtained as described above. It is concentrated to 4 mg/ml in 10 mM Tris-HCl pH 7.5. Initial crystallization screening is done by the sparse matrix crystallization screening kit 1 & 2 from Hampton Research (Jancarik and Kim, 1991; Cudney, *et al.*, 1994). All the screening and crystals growth are accomplished by hanging drops vapor diffusion method at 21°C in VDX multi-well plates with 650 µl reservoir solutions. Drops consisting of 4 µl precipitant buffer from reservoirs and 4 µl protein sample (4 mg/ml) are equilibrated over the well solution for one week.

After extensive screening, plates-like crystals are obtained at two different low salt conditions: (1) 30% PEG 4000, 0.1 M Tris-HCl pH 8.5, 0.2 M $MgCl_2$; (2) 30% PEG 4000, 0.1 M Tris-HCl pH 8.5, 0.2 M NaOAc. 3D cubic-shaped and octahedral crystals also appeared after 3 days at two different high salt conditions: (1) 2.0 M $(NH_4)_2SO_4$, 0.1 M Tris-HCl pH 8.5; (2) 2% PEG 400; 0.1 M Na Hepes pH 7.5, 2.0 M $(NH_4)_2SO_4$. To optimize the crystallization condition, combinations of varied protein and salt concentrations, different molecular weights of PEG, and different pH are screened.

The best crystals formed at the high salt condition is optimized to 2.5% PEG 400, 2.0 M $(NH_4)_2SO_4$, 0.1 M Tris-HCl pH 8.5 at 21°C. They grew to the approximate dimensions of 1.0 × 0.9 × 0.5 mm within five days. The micrographs of Fve crystals are captured by inverted light microscope (Figure 41).

High resolution protein crystals are therefore grown by vapor diffusion from hanging drop at 2.0% PEG 400, 2.0 M $(NH_4)_2SO_4$, 0.1 M Tris-Base, pH 8.5 for 1-2 weeks. Heavy atom derivatives are prepared by soaking the crystals in mother liquor containing 25% glycerol and 1M NaBr. The crystals are flash-frozen at 100 K after a 1-min soak in the heavy atom (Br) solution. SAD data from a derivatized crystal are collected at the National Synchrotron Light Source (NSLS) beam line X12C) at one wavelength (***) around the Br absorption edge. The crystal diffracted to 1.7 Å.

X-ray analysis

The X-ray diffraction intensities from Fve crystals are measured at 100 K on beamline BL9-2 from the Stanford Synchrotron Radiation Laboratory facility with ADSC Quantum-315 CCD detector. Data are collected at a wavelength of 1.07Å. All the data are processed by MOSFLM (Leslie, 1992) and X-ray intensities are scaled with SCALA (CCP4, 1994). Well-ordered diffraction data at 1.4 Å resolution are collected from larger crystals (Figure 42).

Analysis of the collected data (Table 9) indicated that Fve crystals belong to the tetragonal space group $P4_32_12$ with unit cell dimensions of $a = b = 96.92$ Å, $c = 61.42$ Å.

The Matthews parameter (V_M) of these crystals is 2.84 Å^3 per Da and thus the solvent content is 56.37% assuming two molecules of Fve per asymmetric unit (Matthews, 1968). A total of 344079 observations are obtained at 1.4 Å resolution giving approximate 56993 unique reflections (99.7% complete, R_{merge} = 0.047).

X-ray source, beamline	SSRL, BL9-2
Wavelength	1.07Å
Detector distance	99.97mm, Q-315 CCD Detector
Cell angles (°)	90.00, 90.00, 90.00
Unit cell dimensions (Å)	96.92, 96.92, 61.42
Space group	$P4_32_12$
Number of molecules per ASU	2
Number of observed reflections	344079
Number of unique reflections	56993
Solvent (%)	56.37
V_M (Å^{3}Da^{-1})	2.84
Resolution range (Å)	33.5-1.4
Average $I/\sigma(I)$	10.1
R_{merge} [a]	0.047
Completeness (%)	99.7

[a] $R_{merge} = \sum | I_i - < I > | / \sum I_i$, where I_i is the mean intensity of symmetry-related measurements of this reflection.

Table 9. Data Collection and Statistics of Fve Crystal

Data Processing

The SAD data are processed and scaled using DENZO and SCALEPACK from the HKL2000 suite of programs (Otwinowski and Minor, 1997).

The crystal of Fve belongs to the tetragonal space group P43212 and has unit cell dimensions $a = b = 97.12$, $c = 61.41$ and $\alpha = \beta = \gamma = 90.0$. All of the bromine heavy atom positions are located and refined by the program SOLVE at 1.7 Å (Terwilliger and Berendzen, 1999) and solvent flattened map is calculated using RESOLVE (Terwilliger, 2001). The resulting electron density map reveals secondary structure elements and side chains. In principle, it is possible to build an initial model by standard protein map-tracing methods. However, the phases obtained from RESOLVE are directly used in ARP/wARP (Morris et al., 2001) for automated main chains tracing, result in 4 continuous fragments

that contained 97% of model. The rest of the model and side chains are fitted manually using XtalView (McRee, 1999). The refinement is carried out in REFMAC 5 (Murshudov et al., 1999) using resolution range 30.02 - 1.7 and water molecules are picked up by ARP/WARP later in the refinement.

In chain A, C-terminal residue 114 is modeled as Ala residue, whereas in chain B, C-terminal residue 113 and 114 are omitted from the final model, due to the poor interpretable density. The quality of the final model is verified with PROCHECK (Laskowski et al., 1993). However, the Ramachandran plot shows that Lys 14 in both A and B chains is in the disallowed region, although this residue fits very well in the 2fo-fc map.

Example 27. X-Ray Crystallographic Study of Fve: Results

The crystal structure is solved by single anomalous scattering using Br as the heavy-atom, and is refined to a resolution of 1.7 Å. The atomic coordinates are presented in **Appendix C**.

In total, two chains with a total of 226 residues, 16 bromine atoms and 136 solvent molecules are built into a high quality electron density map. Fve comprises almost exclusively of β–sheet structure with an Ig-like fold, which is formed by seven major antiparallel β–strands arranged into two sheets of four (D, E, H and I) and three (B, C and F) strands packed again each other . The N-terminal domain is composed of a α-helix which spans a length of 12 residues from Ala2 to Val13 and a β-sheet (A). The N-terminal serine residue is blocked by an acetyl group the density of which is also observed. Six loops connect the two main β-sheets and one loop connects the N-terminal domain with β–sheet structure. The loop between the β-sheets F and H contains a short β-strand and a 3_{10} helix.

The structure of Fve (Figure 43) reveals that exists as a dimer. This is corroborated experimentally by running Fve on a gel filtration column against standard molecular

weight markers (data not shown). From the structure, there are two extended regions of subunit-subunit interactions: between the two N-terminal α-helical regions (residues 2 to 13) and the β-stranded region (A and A').

The buried side chains of the α-helical regions form a hydrophobic core (Figure 44A), containing residues Ala 2, Leu 5, Leu 9 and Val 13 whereas the side chains of β-strand (A and A') make inter-subunit hydrogen bonds (Figure 44B). These hydrophobic interactions and hydrogen bonds are responsible for dimer formation. The two monomers, A and B chains, of Fve can be closely superimposed: the RMSD between corresponding C_α positions of 112 residues is 0.29 Å (Figure 44C).

Domain Swapping

Domain swapping is a very efficient method of forming oligomers since the interactions within the monomer are reused in the dimer. There is thus no need to evolve a new site on the surface which in one monomer mutually recognizes the corresponding site on the other monomer, since in the domain swapped dimer the recognition requirement has already largely been accounted for (Bennett et al., 1995).

Domain-swapped proteins have a C-interface generally with many specifics interaction, formed between domains linked by a hinge loop (Bennett et al., 1995). In p13suc1, two proline residues, located in the hinge region, have been shown to be essential and control the domain-swapping process (Rousseau et al., 2001).

As shown in Figure 45A, half of the dimer of Fve contains one N-terminal helix, forming a C-interface with hydrophobic core, which is linked to rest of its subunit by a hinge loop, stretching from residue Val 13 to Pro 22. Furthermore, Fve contains a proline residue at the end of the hinge region, which could adopt alternative conformation in the dimer by releasing the tension in the monomer. These observations suggest that domain-swapping could be the mechanism for forming dimer protein from its monomer. The monomer is modeled (Figure 45B).

Structural Similarity with Other Proteins

Fve has no sequence homology to any other non-FIP proteins. However, a search for similar structure in the DALI database (Holm and Sander, 1993) reveals that the protein has a significantly similar fold to 140 proteins but none with the significant sequence similarity to Fve. Among 140 similar fold protein, fibronectin type III family emerged with less topology diversity to Fve β-sandwich fold: the heparin and integrin binding segment of human fibronectin (FN12-15; PDB entry 1FNH), the fragment of human fibronectin type III repeat (FN7-10; 1FNF), The p40 domain of human interlukin-12 (p40; 1F42) and the antibody a6 fragment interferon-gamma receptor alpha chain (IFNγR1-108; 1JRH). An alignment of FN12-15, FN7-10, p40, IFNγR1-108 and Fve on the basis of structural similarity shows topology diversity in the range 11-17, calculated by Topp program (Lu, 1996) (Table 10).

	Name	PDB ID	Z-Score	RMSD	Length of aligned segment	Topological Diversity	Superfamily (Family)	*Species*
1	interleukin-4 receptor alpha chain fragment: b:1-96	1iar-B	5.8	3	78	8.5	Fn III (FNIII)	*Homo sapiens*
2	mhc class ii i-ak: a:82-181	1iak-A	5.8	4.7	83	18.6	Ig (C1)	*Mus musculus*
3	mhc class ii i-ak: b:93-190	1iak-B	5.6	3.5	74	17.8	Ig (C1)	*Mus musculus*
4	igg2a intact antibody - mab23, kappa L chain: a:1-108	1igt-B	5.5	3.8	86	18.4	Ig (V)	*Mus musculus*
5	class ii histocompatibility antigen, HLA-DM: a:94-196	1hdm-B	5.3	4.7	82	18.4	Ig (C1)	*Homo sapiens*
6	fibronectin fragment, heparin & integrin binding segment: a:93-182	1fnh-A	5.3	3	73	11.1	Fn III (FNIII)	*Homo sapiens*
7	ch3 domain of mak33 antibody fragment:chain a	1cqk-A	5.3	3.3	76	18.5	Ig (C1)	*Mus musculus*
8	CD1, beta2-microglobulin and alpha-3 domain: d	1cid	5.3	2.8	76	17.8	Ig (V)	*Rattus rattus*
9	fibronectin fragment, ED-B domain:chain a	2fnb-A	5.2	3.9	72	17	Fn III (FNIII)	*Homo sapiens*
10	hiv-1 gag peptide: a:182-276	1agd-A	5.2	3.8	84	20.1	Ig (C1)	*Homo sapiens*
11	igg1 antibody 32c2 fragment: a:1-110	32c2-A	5.1	5.6	80	19.4	Ig (V)	*Mus musculus*
12	fibronectin repeat 7: 1142-1235	1fnf	5.1	2.7	71	10.8	Fn III (FNIII)	*Homo sapiens*
13	interleukin-12 beta chain fragment: a:88-211	1f42-A	5.1	2.8	70	12.8	Fn III (FNIII)	*Homo sapiens*
14	Mutant growth hormone receptor fragment: b:131-236	1axl-B	5.1	3.2	72	14.7	Fn III (FNIII)	*Homo sapiens*

Table 10

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Each of the applications and patents mentioned in this document, and each document cited or referenced in each of the above applications and patents, including

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Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims.

APPENDIX A: SEQUENCES

Fve is isolated from Golden Needle Mushroom (*Flammulina velutipes*).

ORGANISM: Flammulina velutipes. Eukaryota; Fungi; Basidiomycota; Hymenomycetes; Agaricales; Tricholomataceae; Flammulina.

Fve (Wild type)

```
ATGTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAGATCGACTTCGAC
TACACCCCCAACTGGGGCCGTGGTACCCCAAGCAGCTACATCGACAACCTTACCTTCCCC
AAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGGC
GTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTAC
AACAAGGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGAT
ACCGGCAACTCGGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA
msatsltfqlaylvkkidfdytpnwgrgtpssyidnltfpkvltdkkysyrvvvngsdlg
vesnfavtpsggqtinflqynkgygvadtktiqvfvvipdtgnseeyiiaewkkt
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AGC/AGC/TAC/ATC/GAC/AAC/CTT/ACC/TTC/CCC/AAG/GTT/CTC/ACC/GAC/
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GTC/GAG/TCC/AAC/TTC/GCA/GTG/ACA/CCG/TCC/GGT/GGG/CAG/ACC/ATC/
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ACG/ATT/CAA/GTT/TTC/GTT/GTC/ATT/CCA/GAT/ACC/GGC/AAC/TCG/GAG/
GAG/TAC/ATC/ATC/GCT/GAG/TGG/AAG/AAG/ACT/TGA
```

A Fve (Wild type) sequence may also comprise a sequence as set out above, but lacking the initial methionine (M) in the amino acid sequence, or lacking the initial ATG in the nucleic acid sequence.

GST-Fve (Wild type) Nucleotide Sequence

```
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
TTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAA
TGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGAT
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTT
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAA
AAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
CTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCTCCGCCACGTCGCTCACCTTCCAGCTT
GCCTACTTGGTGAAGAAGATCGACTTCGACTACACCCCCAACTGGGGCCGTGGTACCCCA
AGCAGCTACATCGACAACCTTACCTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTAC
CGCGTCGTGGTCAATGGCTCTGACCTTGGCGTCGAGTCCAACTTCGCAGTGACACCGTCC
```

GGTGGGCAGACCATCAACTTCCTCCAGTACAACAAGGGGTATGGTGTCGCGGACACCAAA
ACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTCGGAGGAGTACATCATCGCT
GAGTGGAAGAAGACTTGA

GST-Fve (Wild type) Amino Acid Sequence

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYID
GDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKV
DFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSSATSLTFQL
AYLVKKIDFDYTPNWGRGTPSSYIDNLTFPKVLTDKKYSYRVVVNGSDLGVESNFAVTPS
GGQTINFLQYNKGYGVADTKTIQVFVVIPDTGNSEEYIIAEWKKT

FVE DELETION MUTANTS

Fve D6-18

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Fve D19-33

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Fve D47-60

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AAG/ACT/TGA

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Fve D61-72

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Fve p55-100

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FVE MUTANTS WITH SINGLE AMINO ACID SUBSTITUTIONS

FveR27A

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FveG28A

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FveT29A

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FUSION PROTEINS OF MAJOR HOUSE DUST MITE ALLERGEN (BLO T 5 OR DER P 2) AND FUNGAL IMMUNOMODULATORY PROTEIN FVE

Blo t 5-Fve (two-in-one chimeric wild type)

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Blo t 5-FveR27A (two-in-one chimeric mutant)

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aactacgaagaggctcaaactctcagcaagatcttgcttaaggatttgaaggaaaccgaacaaaaa
gtgaaggatattcaaacccaaTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAG
ATCGACTTCGACTACACCCCCAACTGGGGC**GCA**GGTACCCCAAGCAGCTACATCGACAACCTTAC
CTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGG
CGTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTACAACAA
GGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTC
GGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA
QEHKPKKDDFRNEFDHLLIEQANHAIEKGEHQLLYLQHQLDELNENKSKELQEKIIRELDVVCAMI
EGAQGALERELKRTDLNILERFNYEEAQTLSKILLKDLKETEQKVKDIQTQsatsltfqlaylvkk
idfdytpnwg**a**gtpssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqyn
kgygvadtktiqvfvvipdtgnseeyiiaewkkt

Blo t 5-FveT29A (two-in-one chimeric mutant)

caagagcacaagccaaagaaggatgatttccgaaacgaattcgatcacttgttgatcgaacaggca
aaccatgctatcgaaaagggagaacatcaattgctttacttgcaacaccaactcgacgaattgaat
gaaaacaagagcaaggaattgcaagagaaaatcattcgagaacttgatgttgtttgcgccatgatc
gaaggagcccaaggagctttggaacgtgaattgaagcgaactgatcttaacattttggaacgattc
aactacgaagaggctcaaactctcagcaagatcttgcttaaggatttgaaggaaaccgaacaaaaa

gtgaaggatattcaaacccaaTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAG
ATCGACTTCGACTACACCCCCAACTGGGGCCGTGGT**GCA**CCAAGCAGCTACATCGACAACCTTAC
CTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGG
CGTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTACAACAA
GGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTC
GGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA
QEHKPKKDDFRNEFDHLLIEQANHAIEKGEHQLLYLQHQLDELNENKSKELQEKIIRELDVVCAMI
EGAQGALERELKRTDLNILERFNYEEAQTLSKILLKDLKETEQKVKDIQTQsatsltfqlaylvkk
idfdytpnwgrg**a**pssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqyn
kgygvadtktiqvfvvipdtgnseeyiiaewkkt

Der p 2-FveR27A (two-in-one chimeric mutant)

gatcaagtcgatgtcaaagattgtgccaatcatgaaatcaaaaaagttttggtaccaggatgccat
ggttcagaaccatgtatcattcatcgtggtaaaccattccaattggaagccgttttcgaagccaac
caaaacacaaaaacggctaaaattgaaatcaaagcctcaatcgatggtttagaagttgatgttccc
ggtatcgatccaaatgcatgccattacatgaaatgcccattggttaaaggacaacaatatgatatt
aaatatacatggaatgttccgaaaattgcaccaaaatctgaaaatgttgtcgtcactgttaaagtt
atgggtgatgatggtgttttggcctgtgctattgctactcatgctaaaatccgcgatTCCGCCACG
TCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAGATCGACTTCGACTACACCCCCAACTGGGGC
GCAGGTACCCCAAGCAGCTACATCGACAACCTTACCTTCCCCAAGGTTCTCACCGACAAAAAATA
CTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGGCGTCGAGTCCAACTTCGCAGTGACACCGTC
CGGTGGGCAGACCATCAACTTCCTCCAGTACAACAAGGGGTATGGTGTCGCGGACACCAAAACGAT
TCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTCGGAGGAGTACATCATCGCTGAGTGGAAGAA
GACTTGA
DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDVP
GIDPNACHYMKCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKVMGDDGVLACAIATHAKIRDsat
sltfqlaylvkkidfdytpnwg**a**gtpssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtp
sggqtinflqynkgygvadtktiqvfvvipdtgnseeyiiaewkkt

Der p 2-FveT29A (two-in-one chimeric mutant)

gatcaagtcgatgtcaaagattgtgccaatcatgaaatcaaaaaagttttggtaccaggatgccat
ggttcagaaccatgtatcattcatcgtggtaaaccattccaattggaagccgttttcgaagccaac
caaaacacaaaaacggctaaaattgaaatcaaagcctcaatcgatggtttagaagttgatgttccc
ggtatcgatccaaatgcatgccattacatgaaatgcccattggttaaaggacaacaatatgatatt
aaatatacatggaatgttccgaaaattgcaccaaaatctgaaaatgttgtcgtcactgttaaagtt
atgggtgatgatggtgttttggcctgtgctattgctactcatgctaaaatccgcgatTCCGCCACG
TCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAGATCGACTTCGACTACACCCCCAACTGGGGC
CGTGGT**GCA**CCAAGCAGCTACATCGACAACCTTACCTTCCCCAAGGTTCTCACCGACAAAAAATA
CTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGGCGTCGAGTCCAACTTCGCAGTGACACCGTC
CGGTGGGCAGACCATCAACTTCCTCCAGTACAACAAGGGGTATGGTGTCGCGGACACCAAAACGAT
TCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTCGGAGGAGTACATCATCGCTGAGTGGAAGAA
GACTTGA
DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDVP
GIDPNACHYMKCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKVMGDDGVLACAIATHAKIRDsat
sltfqlaylvkkidfdytpnwgrg**a**pssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtp
sggqtinflqynkgygvadtktiqvfvvipdtgnseeyiiaewkkt

Blo t 5-Der p 2-FveR27A (three-in-one chimeric mutant)

caagagcacaagccaaagaaggatgatttccgaaacgaattcgatcacttgttgatcgaacaggca
aaccatgctatcgaaaagggagaacatcaattgctttacttgcaacaccaactcgacgaattgaat
gaaaacaagagcaaggaattgcaagagaaaatcattcgagaacttgatgttgtttgcgccatgatc
gaaggagcccaaggagctttggaacgtgaattgaagcgaactgatcttaacattttggaacgattc
aactacgaagaggctcaaactctcagcaagatcttgcttaaggatttgaaggaaaccgaacaaaaa

gtgaaggatattcaaacccaagatcaagtcgatgtcaaagattgtgccaatcatgaaatcaaaaaa
gttttggtaccaggatgccatggttcagaaccatgtatcattcatcgtggtaaaccattccaattg
gaagccgttttcgaagccaaccaaaacacaaaaacggctaaaattgaaatcaaagcctcaatcgat
ggtttagaagttgatgttcccggtatcgatccaaatgcatgccattacatgaaatgcccattggtt
aaaggacaacaatatgatattaaatatacatggaatgttccgaaaattgcaccaaaatctgaaaat
gttgtcgtcactgttaaagttatgggtgatgatggtgttttggcctgtgctattgctactcatgct
aaaatccgcgatTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAGATCGACTTC
GACTACACCCCCAACTGGGGC**GCA**GGTACCCCAAGCAGCTACATCGACAACCTTACCTTCCCCAA
GGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGGCGTCGAGTC
CAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTACAACAAGGGGTATGG
TGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTCGGAGGAGTA
CATCATCGCTGAGTGGAAGAAGACTTGA
QEHKPKKDDFRNEFDHLLIEQANHAIEKGEHQLLYLQHQLDELNENKSKELQEKIIRELDVVCAMI
EGAQGALERELKRTDLNILERFNYEEAQTLSKILLKDLKETEQKVKDIQTQDQVDVKDCANHEIKK
VLVPGCHGSEPCIIHRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDVPGIDPNACHYMKCPLV
KGQQYDIKYTWNVPKIAPKSENVVVTVKVMGDDGVLACAIATHAKIRDsatsltfqlaylvkkidf
dytpnwg**a**gtpssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqynkgy
gvadtktiqvfvvipdtgnseeyiiaewkkt

FUSION PROTEINS OF VIRAL ANTIGEN AND FVE

HPV E7-FveT29A

MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLR
LCVQSTHVDIRTLEDLLMGTLGIVCPICSQKPsatsltfqlaylvkkidfdytpnwgrgapssyid
nltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqynkgygvadtktiqvfvvipdt
gnseeyiiaewkkt
atgcatggagatacacctacattgcatgaatatatgttagatttgcaaccagagacaactgatctc
tactgttatgagcaattaaatgacagctcagaggaggaggatgaaatagatggtccagctggacaa
gcagaaccggacagagcccattacaatattgtaacctttgttgcaagtgtgactctacgcttcgg
ttgtgcgtacaaagcacacacgtagacattcgtactttggaagacctgttaatgggcacactagga
attgtgtgccccatctgttctcagaaaccaTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTG
GTGAAGAAGATCGACTTCGACTACACCCCCAACTGGGGCCGTGGTGCACCAAGCAGCTACATCGAC
AACCTTACCTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCT
GACCTTGGCGTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAG
TACAACAAGGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACC
GGCAACTCGGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA

HCV Core23-FveT29A

Deletion of the 23 amino acids of core antigen from 141-163 amino acid residues leads to increased protein production efficiency

MSTNPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIP
KARQPEGRAWAQPGYPWPLYGNEGLGWAGWLLSPRGSRPSWGPTDPRRRSRNLGKVIDTLTCGFAD
LMGYLPLVYATGNLPGCSFSIFLLALLSCLTIPASAsatsltfqlaylvkkidfdytpnwgrgaps
syidnltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqynkgygvadtktiqvfvv
ipdtgnseeyiiaewkkt
atgagcacgaatcctaaacctcaaagaaaaaccaaacgtaacaccaaccgccgcccacaggacgtc
aagttcccgggcggtggtcagatcgtcggtggagtttacctgttgccgcgcaggggccccaggttg
ggtgtgcgcgcgactaggaagacttccgagcggtcgcaacctcgtggaaggcgacaacctatcccc
aaggctcgccagcccgagggtagggcctgggctcagcccgggtacccctggcccctctatggcaat

gagggcttggggtgggcaggatggctcctgtcaccccgtggctctcggcctagttggggccccacg
gacccccggcgtaggtcgcgcaatttgggtaaggtcatcgataccctcacgtgcggcttcgccgat
ctcatggggtaccttccgctcgtcggcgcaacagggaatctgcccggttgctccttttctatcttc
cttttggctttgctgtcctgtttgaccatcccagcttccgcttatgaagTCCGCCACGTCGCTCAC
CTTCCAGCTTGCCTACTTGGTGAAGAAGATCGACTTCGACTACACCCCCAACTGGGGCCGTGGTGC
ACCAAGCAGCTACATCGACAACCTTACCTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTACCG
CGTCGTGGTCAATGGCTCTGACCTTGGCGTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCA
GACCATCAACTTCCTCCAGTACAACAAGGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTT
CGTTGTCATTCCAGATACCGGCAACTCGGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA

FUSION PROTEINS OF TUMOR-ASSOCIATED ANTIGEN AND FVE

MAGE3-FveT29A

mpleqrsqhckpeegleargealglvgaqapateeqeaasssstlvevtlgevpaaespdppqspq
gasslpttmnyplwsqsyedssnqeeegpstfpdlesefqaalsrkvaelvhfllkyrarepvtk
aemlgsvvgnwqyffpvifskassslqlvfgielmevdpighlyifatclglsydgllgdnqimpk
aglliivlaiiaregdcapeekiweelsvlevfegredsilgdpkklltqhfvqenyleyrqvpgs
dpacyeflwgpralvetsyvkvlhhmvkisggphisypplhewvlregeesatsltfqlaylvkki
dfdytpnwgrgapssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqynkg
ygvadtktiqvfvvipdtgnseeyiiaewkkt
atgcctcttgagcagaggagtcagcactgcaagcctgaagaaggccttgaggcccgaggagaggcc
ctgggcctggtgggtgcgcaggctcctgctactgaggagcaggaggctgcctcctcctcttctact
ctagttgaagtcaccctgggggaggtgcctgctgccgagtcaccagatcctccccagagtcctcag
ggagcctccagcctccccactaccatgaactaccctctctggagccaatcctatgaggactccagc
aaccaagaagaggaggggccaagcaccttccctgacctggagtccgagttccaagcagcactcagt
aggaaggtggccgagttggttcattttctgctcctcaagtatcgagccagggagccggtcacaaag
gcagaaatgctggggagtgtcgtcggaaattggcagtatttctttcctgtgatcttcagcaaagct
tccagttccttgcagctggtctttggcatcgagctgatggaagtggaccccatcggccacttgtac
atctttgccacctgcctgggcctctcctacgatggcctgctgggtgacaatcagatcatgcccaag
gcaggcctcctgataatcgtcctggccataatcgcaagagagggcgactgtgcccctgaggagaaa
atctgggaggagctgagtgtgttagaggtgtttgaggggagggaagacagtatcttgggggatccc
aagaagctgctcacccaacatttcgtgcaggaaaactacctggagtaccggcaggtccccggcagt
gatcctgcatgttatgaattcctgtggggtccaagggccctcgttgaaaccagctatgtgaaagtc
ctgcaccatatggtaaagatcagtggaggacctcacatttcctacccacccctgcatgagtgggtt
ttgagagaggggggaagagTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAGAAGATC
GACTTCGACTACACCCCCAACTGGGGCCGTGGTGCACCAAGCAGCTACATCGACAACCTTACCTTC
CCCAAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTTGGCGTC
GAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTACAACAAGGGG
TATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAACTCGGAG
GAGTACATCATCGCTGAGTGGAAGAAGACTTGA

MART1-FveT29A

mpredahfiygypkkghghsyttaeeaagigiltvilgvllligcwycrrrngyralmdkslhvgt
qcaltrrcpqegfdhrdskvslqekncepvvpnappayeklsaeqspppyspsatsltfqlaylvk
kidfdytpnwgrgapssyidnltfpkvltdkkysyrvvvngsdlgvesnfavtpsggqtinflqyn
kgygvadtktiqvfvvipdtgnseeyiiaewkkt
atgccaagagaagatgctcacttcatctatggttaccccaagaaggggcacggccactcttacacc
acggctgaagaggccgctgggatcggcatcctgacagtgatcctgggagtcttactgctcatcggc
tgttggtattgtagaagacgaaatggatacagagccttgatggataaaagtcttcatgttggcact
caatgtgccttaacaagaagatgcccacaagaagggtttgatcatcgggacagcaaagtgtctctt
caagagaaaaactgtgaacctgtggttcccaatgctccacctgcttatgagaaactctctgcagaa
cagtcaccaccaccttattcaccttCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAG
AAGATCGACTTCGACTACACCCCCAACTGGGGCCGTGGTGCACCAAGCAGCTACATCGACAACCTT
ACCTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTT

GGCGTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTACAAC
AAGGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAAC
TCGGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA

CEA-FveT29A

kltiestpfnvaegkevlllvhnlpqhlfgyswykgervdgnrqiigyvigtqqatpgpaysgrei
iypnaslliqniiqndtgfytlhviksdlvneeatgqfrvypelpkpsissnnskpvedkdavaft
cepetqdatylwwvnnqslpvsprlqlsngnrtltlfnvtrndtasykcetqnpvsarrsdsviln
vlygpdaptisplntsyrsgenlnlschaasnppaqyswfvngtfqqstqelfipnitvnnsgsyt
cqahnsdtglnrttvttitvyaeppkpfitsnnsnpvededavaltcepeiqnttylwwvnnqslp
vsprlqlsndnrtltllsvtrndvgpyecgiqnelsvdhsdpvilnvlygpddptispsytyyrpg
vnlslschaasnppaqyswlidgniqqhtqelfisniteknsglytcqannsasghsrttvktitv
saelpkpsissnnskpvedkdavaftcepeaqnttylwwvngqslpvsprlqlsngnrtltlfnvt
rndarayvcgiqnsvsanrsdpvtldvlygpdtpiisppdssylsganlnlschsasnpspqyswr
ingipqqhtqvlfiakitpnnngtyacfvsnlatgrnnsivksitvsasgtspglsagatvgimig
vlvgvalisatsltfqlaylvkkidfdytpnwgrgapssyidnltfpkvltdkkysyrvvvngsdl
gvesnfavtpsggqtinflqynkgygvadtktiqvfvvipdtgnseeyiiaewkkt
aagctcactattgaatccacgccgttcaatgtcgcagaggggaaggaggtgcttctacttgtccac
aatctgccccagcatctttttggctacagctggtacaaaggtgaaagagtggatggcaaccgtcaa
attataggatatgtaataggaactcaacaagctaccccagggcccgcatacagtggtcgagagata
atataccccaatgcatccctgctgatccagaacatcatccagaatgacacaggattctacacccta
cacgtcataaagtcagatcttgtgaatgaagaagcaactggccagttccgggtatacccggagctg
cccaagccctccatctccagcaacaactccaaacccgtggaggacaaggatgctgtggccttcacc
tgtgaacctgagactcaggacgcaacctacctgtggtgggtaaacaatcagagcctcccggtcagt
cccaggctgcagctgtccaatggcaacaggaccctcactctattcaatgtcacaagaaatgacaca
gcaagctacaaatgtgaaacccagaacccagtgagtgccaggcgcagtgattcagtcatcctgaat
gtcctctatggcccggatgcccccaccatttcccctctaaacacatcttacagatcaggggaaaat
ctgaacctctcctgccatgcagcctctaacccacctgcacagtactcttggtttgtcaatgggact
ttccagcaatccacccaagagctctttatccccaacatcactgtgaataatagtggatcctatacg
tgccaagcccataactcagacactggcctcaataggaccacagtcacgacgatcacagtctatgca
gagccacccaaaccccttcatcaccagcaacaactccaaccccgtggaggatgaggatgctgtagcc
ttaacctgtgaacctgagattcagaacacaacctacctgtggtgggtaaataatcagagcctcccg
gtcagtcccaggctgcagctgtccaatgacaacaggaccctcactctactcagtgtcacaaggaat
gatgtaggaccctatgagtgtggaatccagaacgaattaagtgttgaccacagcgacccagtcatc
ctgaatgtcctctatggcccagacgaccccaccatttcccccctcatacacctattaccgtccaggg
gtgaacctcagcctctcctgccatgcagcctctaacccacctgcacagtattcttggctgattgat
gggaacatccagcaacacacacaagagctctttatctccaacatcactgagaagaacagcggactc
tatacctgccaggccaataactcagccagtggccacagcaggactacagtcaagacaatcacagtc
tctgcggagctgcccaagccctccatctccagcaacaactccaaacccgtggaggacaaggatgct
gtggccttcacctgtgaacctgaggctcagaacacaacctacctgtggtgggtaaatggtcagagc
ctcccagtcagtcccaggctgcagctgtccaatggcaacaggaccctcactctattcaatgtcaca
agaaatgacgcaagagcctatgtatgtggaatccagaactcagtgagtgcaaaccgcagtgaccca
gtcaccctggatgtcctctatgggccggacacccccatcatttcccccccagactcgtcttacctt
tcgggagcgaacctcaacctctcctgccactcggcctctaacccatccccgcagtattcttggcgt
atcaatgggataccgcagcaacacacacaagttctctttatcgccaaaatcacgccaaataataac
gggacctatgcctgttttgtctctaacttggctactggccgcaataattccatagtcaagagcatc
acagtctctgcatctggaacttctcctggtctctcagctggggccactgtcggcatcatgattgga
gtgctggttggggttgctctgataTCCGCCACGTCGCTCACCTTCCAGCTTGCCTACTTGGTGAAG
AAGATCGACTTCGACTACACCCCCAACTGGGGCCGTGGTGCACCAAGCAGCTACATCGACAACCTT
ACCTTCCCCAAGGTTCTCACCGACAAAAAATACTCGTACCGCGTCGTGGTCAATGGCTCTGACCTT
GGCGTCGAGTCCAACTTCGCAGTGACACCGTCCGGTGGGCAGACCATCAACTTCCTCCAGTACAAC
AAGGGGTATGGTGTCGCGGACACCAAAACGATTCAAGTTTTCGTTGTCATTCCAGATACCGGCAAC
TCGGAGGAGTACATCATCGCTGAGTGGAAGAAGACTTGA

PRIMERS FOR CONSTRUCTION OF FVE DELETION MUTANTS

Fd6-18F (36 mer)

5′-ggA/TCC/TCC/gCC/ACg/TCg/TTC/gAC/TAC/ACC/CCC/AAC- 3′

Fd6-18R (36 mer)

5′-gTT/ggg/ggT/gTA/gTC/gAA/CgA/CgT/ggC/ggA/ggA/TCC- 3′

Fd19-33F (36 mer)

5′-TTg/gTg/AAg/AAg/ATC/gAC/ATC/gAC/AAC/CTT/ACC/TTC- 3′

Fd19-33R (36 mer)

5′-gAA/ggT/AAg/gTT/gTC/gAT/gTC/gAT/CTT/CTT/CAC/CAA- 3′

Fd34-46F (36 mer)

5′-ggT/ACC/CCA/AgC/AgC/TAC/AAA/TAC/TCg/TAC/CgC/gTC- 3′

Fd34-46R (36 mer)

5′-gAC/gCg/gTA/CgA/gTA/TTT/gTA/gCT/gCT/Tgg/ggT/ACC- 3′

Fd47-60F (36 mer)

5′-AAg/gTT/CTC/ACC/gAC/AAA/gTC/gAg/TCC/AAC/TTC/gCA- 3′

Fd47-60R (36 mer)

5′-TgC/gAA/gTT/ggA/CTC/gAC/TTT/gTC/ggT/gAg/AAC/CTT- 3′

Fd61-72F (36 mer)

5′-AAT/ggC/TCT/gAC/CTT/ggC/CAg/ACC/ATC/AAC/TTC/CTC- 3′

Fd61-72R (36 mer)

5′-gAg/gAA/gTT/gAT/ggT/CTg/gCC/AAg/gTC/AgA/gCC/ATT- 3′

Fd73-84F (36 mer)

5′-gTg/ACA/CCg/TCC/ggT/ggg/ggT/gTC/gCg/gAC/ACC/AAA- 3′

Fd73-84R (36 mer)

5′-TTT/ggT/gTC/CgC/gAC/ACC/CCC/ACC/ggA/Cgg/TgT/CAC- 3′

Fd85-97F (36 mer)

5′-CAg/TAC/AAC/AAg/ggg/TAT/ATT/CCA/gAT/ACC/ggC/AAC- 3′

Fd85-97R (36 mer)

5′-gTT/gCC/ggT/ATC/Tgg/AAT/ATA/CCC/CTT/gTT/gTA/CTg- 3′

Fd98-106F (36 mer)

5′-ATT/CAA/gTT/TTC/gTT/gTC/TAC/ATC/ATC/gCT/gAg/Tgg- 3′

Fd98-106R (36 mer)

5′-CCA/CTC/AgC/gAT/gAT/gTA/gAC/AAC/gAA/AAC/TTg/AAT- 3′

Fd107-115R (39 mer)

5′-gAT/gCA/ACT/gAA/TTC/TTA/TTA/CTC/CTC/CgA/gTT/gCC/ggT- 3′

PRIMERS FOR CONSTRUCTION OF LARGE FRAGMENT DELETION OF FVE

d(61-97)-F (36mer)

5′-/AAT/ggC/TCT/gAC/CTT/ggC/ATT/CCA/gAT/ACC/ggC/AAC/-3′

d(61-97)-R (36mer)

5′-/gTT/gCC/ggT/ATC/Tgg/AAT/gCC/AAg/gTC/AgA/gCC/ATT/-3′

PRIMERS FOR CONSTRUCTION OF SMALL FRAGMENT OF FVE (FROM 55AA TO 100AA)

[Fv55-100]-F (48mer)

5′-
/gTT/CCg/CgT/ggA/TCC/ATC/gAA/ggT/CgT/AAT/ggC/TCT/gAC/CTT/ggC/gTC/-
3′

[Fv55-100]-R (42mer)

5′-/gAT/gCA/ACT/gAA/TTC/TTA/TCA/ATC/Tgg/AAT/gAC/AAC/gAA/AAC/-3′

PRIMERS FOR CONSTRUCTION OF POINT MUTANTS OF FVE

F(R27A)-F (27 mer)

5′- CCC/AAC/Tgg/ggC/gCA/ggT/ACC/CCA/AgC - 3′

F(R27A)-R (27 mer)

5′- gCT/Tgg/ggT/ACC/TgC/gCC/CCA/gTT/ggg - 3′

F(G28A)-F (27 mer)

5′- AAC/Tgg/ggC/CgT/gCA/ACC/CCA/AgC/AgC - 3′

F(G28A)-R (27 mer)

5′- gCT/gCT/Tgg/ggT/TgC/ACg/gCC/CCA/gTT - 3′

F(T29A)-F (27 mer)

5′- Tgg/ggC/CgT/ggT/gCA/CCA/AgC/AgC/TAC - 3′

F(T29A)-R (27 mer)

5′- gTA/gCT/gCT/Tgg/TgC/ACC/ACg/gCC/CCA - 3′

PRIMERS FOR BLO T 5-FVE FUSION PROTEIN

Bt5Fv-F (36mer)

5′-/AAg/gAT/ATT/CAA/ACC/CAA/TCC/gCC/ACg/TCg/CTC/ACC/-3′

Bt5Fv-R (36mer)

5′-/ggT/gAg/CgA/CgT/ggC/ggA/TTg/ggT/TTg/AAT/ATC/CTT/-3′

PRIMERS FOR DER P 2-FVE FUSION PROTEIN

Dp2Fv-F (36mer)

5′-/CAT/gCT/AAA/ATC/CgC/gAT/TCC/gCC/ACg/TCg/CTC/ACC-3′

Dp2Fv-R (36mer)

5′-/ggT/gAg/CgA/CgT/ggC/ggA/ATC/gCg/gAT/TTT/AgC/ATg-3′

PRIMERS FOR BLO T 5-DER P 2-FVE FUSION PROTEIN

Bt5Dp2-F (36mer)

5′-/aag/gat/att/caa/acc/caa/gat/caa/gtc/gat/gtc/aaa/-3′

Bt5Dp2-R (36mer)

5′-/ttt/gac/atc/gac/ttg/atc/ttg/ggt/ttg/aat/atc/ctt/-3′

APPENDIX B: FVE FRAGMENTS (RGT TRIPLET HIGHLIGHTED)

Fragment Number	Residues	Sequence
1	24-28	WGRGT
2	25-29	GRGTP
3	26-30	RGTPS
4	27-31	GTPSS
5	28-32	TPSSY
6	23-28	NWGRGT
7	24-29	WGRGTP
8	25-30	GRGTPS
9	26-31	RGTPSS
10	27-32	GTPSSY
11	28-33	TPSSYI
12	22-28	PNWGRGT
13	23-29	NWGRGTP
14	24-30	WGRGTPS
15	25-31	GRGTPSS
16	26-32	RGTPSSY
17	27-33	GTPSSYI
18	28-34	TPSSYID
19	21-28	TPNWGRGT
20	22-29	PNWGRGTP
21	23-30	NWGRGTPS
22	24-31	WGRGTPSS
23	25-32	GRGTPSSY
24	26-33	RGTPSSYI
25	27-34	GTPSSYID
26	28-35	TPSSYIDN
27	20-28	YTPNWGRGT
28	21-29	TPNWGRGTP
29	22-30	PNWGRGTPS
30	23-31	NWGRGTPSS
31	24-32	WGRGTPSSY
32	25-33	GRGTPSSYI
33	26-34	RGTPSSYID
34	27-35	GTPSSYIDN
35	28-36	TPSSYIDNL
36	19-28	DYTPNWGRGT
37	20-29	YTPNWGRGTP
38	21-30	TPNWGRGTPS
39	22-31	PNWGRGTPSS
40	23-32	NWGRGTPSSY

Fragment Number	Residues	Sequence
41	24-33	WGRGTPSSYI
42	25-34	GRGTPSSYID
43	26-35	RGTPSSYIDN
44	27-36	GTPSSYIDNL
45	28-37	TPSSYIDNLT
46	18-28	FDYTPNWGRGT
47	19-29	DYTPNWGRGTP
48	20-30	YTPNWGRGTPS
49	21-31	TPNWGRGTPSS
50	22-32	PNWGRGTPSSY
51	23-33	NWGRGTPSSYI
52	24-34	WGRGTPSSYID
53	25-35	GRGTPSSYIDN
54	26-36	RGTPSSYIDNL
55	27-37	GTPSSYIDNLT
56	28-38	TPSSYIDNLTF
57	17-28	DFDYTPNWGRGT
58	18-29	FDYTPNWGRGTP
59	19-30	DYTPNWGRGTPS
60	20-31	YTPNWGRGTPSS
61	21-32	TPNWGRGTPSSY
62	22-33	PNWGRGTPSSYI
63	23-34	NWGRGTPSSYID
64	24-35	WGRGTPSSYIDN
65	25-36	GRGTPSSYIDNL
66	26-37	RGTPSSYIDNLT
67	27-38	GTPSSYIDNLTF
68	28-39	TPSSYIDNLTFP
69	16-28	IDFDYTPNWGRGT
70	17-29	DFDYTPNWGRGTP
71	18-30	FDYTPNWGRGTPS
72	19-31	DYTPNWGRGTPSS
73	20-32	YTPNWGRGTPSSY
74	21-33	TPNWGRGTPSSYI
75	22-34	PNWGRGTPSSYID
76	23-35	NWGRGTPSSYIDN
77	24-36	WGRGTPSSYIDNL
78	25-37	GRGTPSSYIDNLT
79	26-38	RGTPSSYIDNLTF
80	27-39	GTPSSYIDNLTFP
81	28-40	TPSSYIDNLTFPK
82	15-28	KIDFDYTPNWGRGT
83	16-29	IDFDYTPNWGRGTP

Fragment Number	Residues	Sequence
84	17-30	DFDYTPNWGRGTPS
85	18-31	FDYTPNWGRGTPSS
86	19-32	DYTPNWGRGTPSSY
87	20-33	YTPNWGRGTPSSYI
88	21-34	TPNWGRGTPSSYID
89	22-35	PNWGRGTPSSYIDN
90	23-36	NWGRGTPSSYIDNL
91	24-37	WGRGTPSSYIDNLT
92	25-38	GRGTPSSYIDNLTF
93	26-39	RGTPSSYIDNLTFP
94	27-40	GTPSSYIDNLTFPK
95	28-41	TPSSYIDNLTFPKV
96	14-28	KKIDFDYTPNWGRGT
97	15-29	KIDFDYTPNWGRGTP
98	16-30	IDFDYTPNWGRGTPS
99	17-31	DFDYTPNWGRGTPSS
100	18-32	FDYTPNWGRGTPSSY
101	19-33	DYTPNWGRGTPSSYI
102	20-34	YTPNWGRGTPSSYID
103	21-35	TPNWGRGTPSSYIDN
104	22-36	PNWGRGTPSSYIDNL
105	23-37	NWGRGTPSSYIDNLT
106	24-38	WGRGTPSSYIDNLTF
107	25-39	GRGTPSSYIDNLTFP
108	26-40	RGTPSSYIDNLTFPK
109	27-41	GTPSSYIDNLTFPKV
110	28-42	TPSSYIDNLTFPKVL
111	13-28	VKKIDFDYTPNWGRGT
112	14-29	KKIDFDYTPNWGRGTP
113	15-30	KIDFDYTPNWGRGTPS
114	16-31	IDFDYTPNWGRGTPSS
115	17-32	DFDYTPNWGRGTPSSY
116	18-33	FDYTPNWGRGTPSSYI
117	19-34	DYTPNWGRGTPSSYID
118	20-35	YTPNWGRGTPSSYIDN
119	21-36	TPNWGRGTPSSYIDNL
120	22-37	PNWGRGTPSSYIDNLT
121	23-38	NWGRGTPSSYIDNLTF
122	24-39	WGRGTPSSYIDNLTFP
123	25-40	GRGTPSSYIDNLTFPK
124	26-41	RGTPSSYIDNLTFPKV
125	27-42	GTPSSYIDNLTFPKVL
126	28-43	TPSSYIDNLTFPKVLT

Fragment Number	Residues	Sequence
127	12-28	LVKKIDFDYTPNWGRGT
128	13-29	VKKIDFDYTPNWGRGTP
129	14-30	KKIDFDYTPNWGRGTPS
130	15-31	KIDFDYTPNWGRGTPSS
131	16-32	IDFDYTPNWGRGTPSSY
132	17-33	DFDYTPNWGRGTPSSYI
133	18-34	FDYTPNWGRGTPSSYID
134	19-35	DYTPNWGRGTPSSYIDN
135	20-36	YTPNWGRGTPSSYIDNL
136	21-37	TPNWGRGTPSSYIDNLT
137	22-38	PNWGRGTPSSYIDNLTF
138	23-39	NWGRGTPSSYIDNLTFP
139	24-40	WGRGTPSSYIDNLTFPK
140	25-41	GRGTPSSYIDNLTFPKV
141	26-42	RGTPSSYIDNLTFPKVL
142	27-43	GTPSSYIDNLTFPKVLT
143	28-44	TPSSYIDNLTFPKVLTD
144	11-28	YLVKKIDFDYTPNWGRGT
145	12-29	LVKKIDFDYTPNWGRGTP
146	13-30	VKKIDFDYTPNWGRGTPS
147	14-31	KKIDFDYTPNWGRGTPSS
148	15-32	KIDFDYTPNWGRGTPSSY
149	16-33	IDFDYTPNWGRGTPSSYI
150	17-34	DFDYTPNWGRGTPSSYID
151	18-35	FDYTPNWGRGTPSSYIDN
152	19-36	DYTPNWGRGTPSSYIDNL
153	20-37	YTPNWGRGTPSSYIDNLT
154	21-38	TPNWGRGTPSSYIDNLTF
155	22-39	PNWGRGTPSSYIDNLTFP
156	23-40	NWGRGTPSSYIDNLTFPK
157	24-41	WGRGTPSSYIDNLTFPKV
158	25-42	GRGTPSSYIDNLTFPKVL
159	26-43	RGTPSSYIDNLTFPKVLT
160	27-44	GTPSSYIDNLTFPKVLTD
161	28-45	TPSSYIDNLTFPKVLTDK
162	10-28	AYLVKKIDFDYTPNWGRGT
163	11-29	YLVKKIDFDYTPNWGRGTP
164	12-30	LVKKIDFDYTPNWGRGTPS
165	13-31	VKKIDFDYTPNWGRGTPSS
166	14-32	KKIDFDYTPNWGRGTPSSY
167	15-33	KIDFDYTPNWGRGTPSSYI
168	16-34	IDFDYTPNWGRGTPSSYID
169	17-35	DFDYTPNWGRGTPSSYIDN

Fragment Number	Residues	Sequence
170	18-36	FDYTPNWGRGTPSSYIDNL
171	19-37	DYTPNWGRGTPSSYIDNLT
172	20-38	YTPNWGRGTPSSYIDNLTF
173	21-39	TPNWGRGTPSSYIDNLTFP
174	22-40	PNWGRGTPSSYIDNLTFPK
175	23-41	NWGRGTPSSYIDNLTFPKV
176	24-42	WGRGTPSSYIDNLTFPKVL
177	25-43	GRGTPSSYIDNLTFPKVLT
178	26-44	RGTPSSYIDNLTFPKVLTD
179	27-45	GTPSSYIDNLTFPKVLTDK
180	28-46	TPSSYIDNLTFPKVLTDKK
181	9-28	LAYLVKKIDFDYTPNWGRGT
182	10-29	AYLVKKIDFDYTPNWGRGTP
183	11-30	YLVKKIDFDYTPNWGRGTPS
184	12-31	LVKKIDFDYTPNWGRGTPSS
185	13-32	VKKIDFDYTPNWGRGTPSSY
186	14-33	KKIDFDYTPNWGRGTPSSYI
187	15-34	KIDFDYTPNWGRGTPSSYID
188	16-35	IDFDYTPNWGRGTPSSYIDN
189	17-36	DFDYTPNWGRGTPSSYIDNL
190	18-37	FDYTPNWGRGTPSSYIDNLT
191	19-38	DYTPNWGRGTPSSYIDNLTF
192	20-39	YTPNWGRGTPSSYIDNLTFP
193	21-40	TPNWGRGTPSSYIDNLTFPK
194	22-41	PNWGRGTPSSYIDNLTFPKV
195	23-42	NWGRGTPSSYIDNLTFPKVL
196	24-43	WGRGTPSSYIDNLTFPKVLT
197	25-44	GRGTPSSYIDNLTFPKVLTD
198	26-45	RGTPSSYIDNLTFPKVLTDK
199	27-46	GTPSSYIDNLTFPKVLTDKK
200	28-47	TPSSYIDNLTFPKVLTDKKY
201	8-28	QLAYLVKKIDFDYTPNWGRGT
202	9-29	LAYLVKKIDFDYTPNWGRGTP
203	10-30	AYLVKKIDFDYTPNWGRGTPS
204	11-31	YLVKKIDFDYTPNWGRGTPSS
205	12-32	LVKKIDFDYTPNWGRGTPSSY
206	13-33	VKKIDFDYTPNWGRGTPSSYI
207	14-34	KKIDFDYTPNWGRGTPSSYID
208	15-35	KIDFDYTPNWGRGTPSSYIDN
209	16-36	IDFDYTPNWGRGTPSSYIDNL
210	17-37	DFDYTPNWGRGTPSSYIDNLT
211	18-38	FDYTPNWGRGTPSSYIDNLTF
212	19-39	DYTPNWGRGTPSSYIDNLTFP

Fragment Number	Residues	Sequence
213	20-40	YTPNWGRGTPSSYIDNLTFPK
214	21-41	TPNWGRGTPSSYIDNLTFPKV
215	22-42	PNWGRGTPSSYIDNLTFPKVL
216	23-43	NWGRGTPSSYIDNLTFPKVLT
217	24-44	WGRGTPSSYIDNLTFPKVLTD
218	25-45	GRGTPSSYIDNLTFPKVLTDK
219	26-46	RGTPSSYIDNLTFPKVLTDKK
220	27-47	GTPSSYIDNLTFPKVLTDKKY
221	28-48	TPSSYIDNLTFPKVLTDKKYS
222	7-28	FQLAYLVKKIDFDYTPNWGRGT
223	8-29	QLAYLVKKIDFDYTPNWGRGTP
224	9-30	LAYLVKKIDFDYTPNWGRGTPS
225	10-31	AYLVKKIDFDYTPNWGRGTPSS
226	11-32	YLVKKIDFDYTPNWGRGTPSSY
227	12-33	LVKKIDFDYTPNWGRGTPSSYI
228	13-34	VKKIDFDYTPNWGRGTPSSYID
229	14-35	KKIDFDYTPNWGRGTPSSYIDN
230	15-36	KIDFDYTPNWGRGTPSSYIDNL
231	16-37	IDFDYTPNWGRGTPSSYIDNLT
232	17-38	DFDYTPNWGRGTPSSYIDNLTF
233	18-39	FDYTPNWGRGTPSSYIDNLTFP
234	19-40	DYTPNWGRGTPSSYIDNLTFPK
235	20-41	YTPNWGRGTPSSYIDNLTFPKV
236	21-42	TPNWGRGTPSSYIDNLTFPKVL
237	22-43	PNWGRGTPSSYIDNLTFPKVLT
238	23-44	NWGRGTPSSYIDNLTFPKVLTD
239	24-45	WGRGTPSSYIDNLTFPKVLTDK
240	25-46	GRGTPSSYIDNLTFPKVLTDKK
241	26-47	RGTPSSYIDNLTFPKVLTDKKY
242	27-48	GTPSSYIDNLTFPKVLTDKKYS
243	28-49	TPSSYIDNLTFPKVLTDKKYSY
244	6-28	TFQLAYLVKKIDFDYTPNWGRGT
245	7-29	FQLAYLVKKIDFDYTPNWGRGTP
246	8-30	QLAYLVKKIDFDYTPNWGRGTPS
247	9-31	LAYLVKKIDFDYTPNWGRGTPSS
248	10-32	AYLVKKIDFDYTPNWGRGTPSSY
249	11-33	YLVKKIDFDYTPNWGRGTPSSYI
250	12-34	LVKKIDFDYTPNWGRGTPSSYID
251	13-35	VKKIDFDYTPNWGRGTPSSYIDN
252	14-36	KKIDFDYTPNWGRGTPSSYIDNL
253	15-37	KIDFDYTPNWGRGTPSSYIDNLT
254	16-38	IDFDYTPNWGRGTPSSYIDNLTF
255	17-39	DFDYTPNWGRGTPSSYIDNLTFP

Fragment Number	Residues	Sequence
256	18-40	FDYTPNWGRGTPSSYIDNLTFPK
257	19-41	DYTPNWGRGTPSSYIDNLTFPKV
258	20-42	YTPNWGRGTPSSYIDNLTFPKVL
259	21-43	TPNWGRGTPSSYIDNLTFPKVLT
260	22-44	PNWGRGTPSSYIDNLTFPKVLTD
261	23-45	NWGRGTPSSYIDNLTFPKVLTDK
262	24-46	WGRGTPSSYIDNLTFPKVLTDKK
263	25-47	GRGTPSSYIDNLTFPKVLTDKKY
264	26-48	RGTPSSYIDNLTFPKVLTDKKYS
265	27-49	GTPSSYIDNLTFPKVLTDKKYSY
266	28-50	TPSSYIDNLTFPKVLTDKKYSYR
267	5-28	LTFQLAYLVKKIDFDYTPNWGRGT
268	6-29	TFQLAYLVKKIDFDYTPNWGRGTP
269	7-30	FQLAYLVKKIDFDYTPNWGRGTPS
270	8-31	QLAYLVKKIDFDYTPNWGRGTPSS
271	9-32	LAYLVKKIDFDYTPNWGRGTPSSY
272	10-33	AYLVKKIDFDYTPNWGRGTPSSYI
273	11-34	YLVKKIDFDYTPNWGRGTPSSYID
274	12-35	LVKKIDFDYTPNWGRGTPSSYIDN
275	13-36	VKKIDFDYTPNWGRGTPSSYIDNL
276	14-37	KKIDFDYTPNWGRGTPSSYIDNLT
277	15-38	KIDFDYTPNWGRGTPSSYIDNLTF
278	16-39	IDFDYTPNWGRGTPSSYIDNLTFP
279	17-40	DFDYTPNWGRGTPSSYIDNLTFPK
280	18-41	FDYTPNWGRGTPSSYIDNLTFPKV
281	19-42	DYTPNWGRGTPSSYIDNLTFPKVL
282	20-43	YTPNWGRGTPSSYIDNLTFPKVLT
283	21-44	TPNWGRGTPSSYIDNLTFPKVLTD
284	22-45	PNWGRGTPSSYIDNLTFPKVLTDK
285	23-46	NWGRGTPSSYIDNLTFPKVLTDKK
286	24-47	WGRGTPSSYIDNLTFPKVLTDKKY
287	25-48	GRGTPSSYIDNLTFPKVLTDKKYS
288	26-49	RGTPSSYIDNLTFPKVLTDKKYSY
289	27-50	GTPSSYIDNLTFPKVLTDKKYSYR
290	28-51	TPSSYIDNLTFPKVLTDKKYSYRV
291	4-28	SLTFQLAYLVKKIDFDYTPNWGRGT
292	5-29	LTFQLAYLVKKIDFDYTPNWGRGTP
293	6-30	TFQLAYLVKKIDFDYTPNWGRGTPS
294	7-31	FQLAYLVKKIDFDYTPNWGRGTPSS
295	8-32	QLAYLVKKIDFDYTPNWGRGTPSSY
296	9-33	LAYLVKKIDFDYTPNWGRGTPSSYI
297	10-34	AYLVKKIDFDYTPNWGRGTPSSYID
298	11-35	YLVKKIDFDYTPNWGRGTPSSYIDN

Fragment Number	Residues	Sequence
299	12-36	LVKKIDFDYTPNWGRGTPSSYIDNL
300	13-37	VKKIDFDYTPNWGRGTPSSYIDNLT
301	14-38	KKIDFDYTPNWGRGTPSSYIDNLTF
302	15-39	KIDFDYTPNWGRGTPSSYIDNLTFP
303	16-40	IDFDYTPNWGRGTPSSYIDNLTFPK
304	17-41	DFDYTPNWGRGTPSSYIDNLTFPKV
305	18-42	FDYTPNWGRGTPSSYIDNLTFPKVL
306	19-43	DYTPNWGRGTPSSYIDNLTFPKVLT
307	20-44	YTPNWGRGTPSSYIDNLTFPKVLTD
308	21-45	TPNWGRGTPSSYIDNLTFPKVLTDK
309	22-46	PNWGRGTPSSYIDNLTFPKVLTDKK
310	23-47	NWGRGTPSSYIDNLTFPKVLTDKKY
311	24-48	WGRGTPSSYIDNLTFPKVLTDKKYS
312	25-49	GRGTPSSYIDNLTFPKVLTDKKYSY
313	26-50	RGTPSSYIDNLTFPKVLTDKKYSYR
314	27-51	GTPSSYIDNLTFPKVLTDKKYSYRV
315	28-52	TPSSYIDNLTFPKVLTDKKYSYRVV
316	3-28	TSLTFQLAYLVKKIDFDYTPNWGRGT
317	4-29	SLTFQLAYLVKKIDFDYTPNWGRGTP
318	5-30	LTFQLAYLVKKIDFDYTPNWGRGTPS
319	6-31	TFQLAYLVKKIDFDYTPNWGRGTPSS
320	7-32	FQLAYLVKKIDFDYTPNWGRGTPSSY
321	8-33	QLAYLVKKIDFDYTPNWGRGTPSSYI
322	9-34	LAYLVKKIDFDYTPNWGRGTPSSYID
323	10-35	AYLVKKIDFDYTPNWGRGTPSSYIDN
324	11-36	YLVKKIDFDYTPNWGRGTPSSYIDNL
325	12-37	LVKKIDFDYTPNWGRGTPSSYIDNLT
326	13-38	VKKIDFDYTPNWGRGTPSSYIDNLTF
327	14-39	KKIDFDYTPNWGRGTPSSYIDNLTFP
328	15-40	KIDFDYTPNWGRGTPSSYIDNLTFPK
329	16-41	IDFDYTPNWGRGTPSSYIDNLTFPKV
330	17-42	DFDYTPNWGRGTPSSYIDNLTFPKVL
331	18-43	FDYTPNWGRGTPSSYIDNLTFPKVLT
332	19-44	DYTPNWGRGTPSSYIDNLTFPKVLTD
333	20-45	YTPNWGRGTPSSYIDNLTFPKVLTDK
334	21-46	TPNWGRGTPSSYIDNLTFPKVLTDKK
335	22-47	PNWGRGTPSSYIDNLTFPKVLTDKKY
336	23-48	NWGRGTPSSYIDNLTFPKVLTDKKYS
337	24-49	WGRGTPSSYIDNLTFPKVLTDKKYSY
338	25-50	GRGTPSSYIDNLTFPKVLTDKKYSYR
339	26-51	RGTPSSYIDNLTFPKVLTDKKYSYRV
340	27-52	GTPSSYIDNLTFPKVLTDKKYSYRVV
341	28-53	TPSSYIDNLTFPKVLTDKKYSYRVVV

Fragment Number	Residues	Sequence
342	2-28	ATSLTFQLAYLVKKIDFDYTPNWGRGT
343	3-29	TSLTFQLAYLVKKIDFDYTPNWGRGTP
344	4-30	SLTFQLAYLVKKIDFDYTPNWGRGTPS
345	5-31	LTFQLAYLVKKIDFDYTPNWGRGTPSS
346	6-32	TFQLAYLVKKIDFDYTPNWGRGTPSSY
347	7-33	FQLAYLVKKIDFDYTPNWGRGTPSSYI
348	8-34	QLAYLVKKIDFDYTPNWGRGTPSSYID
349	9-35	LAYLVKKIDFDYTPNWGRGTPSSYIDN
350	10-36	AYLVKKIDFDYTPNWGRGTPSSYIDNL
351	11-37	YLVKKIDFDYTPNWGRGTPSSYIDNLT
352	12-38	LVKKIDFDYTPNWGRGTPSSYIDNLTF
353	13-39	VKKIDFDYTPNWGRGTPSSYIDNLTFP
354	14-40	KKIDFDYTPNWGRGTPSSYIDNLTFPK
355	15-41	KIDFDYTPNWGRGTPSSYIDNLTFPKV
356	16-42	IDFDYTPNWGRGTPSSYIDNLTFPKVL
357	17-43	DFDYTPNWGRGTPSSYIDNLTFPKVLT
358	18-44	FDYTPNWGRGTPSSYIDNLTFPKVLTD
359	19-45	DYTPNWGRGTPSSYIDNLTFPKVLTDK
360	20-46	YTPNWGRGTPSSYIDNLTFPKVLTDKK
361	21-47	TPNWGRGTPSSYIDNLTFPKVLTDKKY
362	22-48	PNWGRGTPSSYIDNLTFPKVLTDKKYS
363	23-49	NWGRGTPSSYIDNLTFPKVLTDKKYSY
364	24-50	WGRGTPSSYIDNLTFPKVLTDKKYSYR
365	25-51	GRGTPSSYIDNLTFPKVLTDKKYSYRV
366	26-52	RGTPSSYIDNLTFPKVLTDKKYSYRVV
367	27-53	GTPSSYIDNLTFPKVLTDKKYSYRVVV
368	28-54	TPSSYIDNLTFPKVLTDKKYSYRVVVN
369	1-28	SATSLTFQLAYLVKKIDFDYTPNWGRGT
370	2-29	ATSLTFQLAYLVKKIDFDYTPNWGRGTP
371	3-30	TSLTFQLAYLVKKIDFDYTPNWGRGTPS
372	4-31	SLTFQLAYLVKKIDFDYTPNWGRGTPSS
373	5-32	LTFQLAYLVKKIDFDYTPNWGRGTPSSY
374	6-33	TFQLAYLVKKIDFDYTPNWGRGTPSSYI
375	7-34	FQLAYLVKKIDFDYTPNWGRGTPSSYID
376	8-35	QLAYLVKKIDFDYTPNWGRGTPSSYIDN
377	9-36	LAYLVKKIDFDYTPNWGRGTPSSYIDNL
378	10-37	AYLVKKIDFDYTPNWGRGTPSSYIDNLT
379	11-38	YLVKKIDFDYTPNWGRGTPSSYIDNLTF
380	12-39	LVKKIDFDYTPNWGRGTPSSYIDNLTFP
381	13-40	VKKIDFDYTPNWGRGTPSSYIDNLTFPK
382	14-41	KKIDFDYTPNWGRGTPSSYIDNLTFPKV
383	15-42	KIDFDYTPNWGRGTPSSYIDNLTFPKVL
384	16-43	IDFDYTPNWGRGTPSSYIDNLTFPKVLT

Fragment Number	Residues	Sequence
385	17-44	DFDYTPNWGRGTPSSYIDNLTFPKVLTD
386	18-45	FDYTPNWGRGTPSSYIDNLTFPKVLTDK
387	19-46	DYTPNWGRGTPSSYIDNLTFPKVLTDKK
388	20-47	YTPNWGRGTPSSYIDNLTFPKVLTDKKY
389	21-48	TPNWGRGTPSSYIDNLTFPKVLTDKKYS
390	22-49	PNWGRGTPSSYIDNLTFPKVLTDKKYSY
391	23-50	NWGRGTPSSYIDNLTFPKVLTDKKYSYR
392	24-51	WGRGTPSSYIDNLTFPKVLTDKKYSYRV
393	25-52	GRGTPSSYIDNLTFPKVLTDKKYSYRVV
394	26-53	RGTPSSYIDNLTFPKVLTDKKYSYRVVV
395	27-54	GTPSSYIDNLTFPKVLTDKKYSYRVVVN
396	28-55	TPSSYIDNLTFPKVLTDKKYSYRVVVNG

APPENDIX C: CRYSTAL COORDINATES OF FVE PROTEIN

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HEADER    ----                                                  XX-XXX-XX    xxxx
COMPND    ---
REMARK   3
REMARK   3 REFINEMENT.
REMARK   3   PROGRAM     : REFMAC 5.0
REMARK   3   AUTHORS     : MURSHUDOV,VAGIN,DODSON
REMARK   3
REMARK   3    REFINEMENT TARGET : MAXIMUM LIKELIHOOD
REMARK   3
REMARK   3  DATA USED IN REFINEMENT.
REMARK   3   RESOLUTION RANGE HIGH (ANGSTROMS) :   1.70
REMARK   3   RESOLUTION RANGE LOW  (ANGSTROMS) :  30.02
REMARK   3   DATA CUTOFF            (SIGMA(F)) : NONE
REMARK   3   COMPLETENESS FOR RANGE        (%) :  98.80
REMARK   3   NUMBER OF REFLECTIONS             :   30783
REMARK   3
REMARK   3  FIT TO DATA USED IN REFINEMENT.
REMARK   3   CROSS-VALIDATION METHOD           : THROUGHOUT
REMARK   3   FREE R VALUE TEST SET SELECTION   : RANDOM
REMARK   3   R VALUE     (WORKING + TEST SET) : 0.18358
REMARK   3   R VALUE              (WORKING SET) :  0.18218
REMARK   3   FREE R VALUE                       :  0.21016
REMARK   3   FREE R VALUE TEST SET SIZE   (%) :  5.1
REMARK   3   FREE R VALUE TEST SET COUNT      :  1650
REMARK   3
REMARK   3  FIT IN THE HIGHEST RESOLUTION BIN.
REMARK   3   TOTAL NUMBER OF BINS USED           :      20
REMARK   3   BIN RESOLUTION RANGE HIGH           :   1.701
REMARK   3   BIN RESOLUTION RANGE LOW            :   1.745
REMARK   3   REFLECTION IN BIN     (WORKING SET) :    2183
REMARK   3   BIN R VALUE           (WORKING SET) :   0.160
REMARK   3   BIN FREE R VALUE SET COUNT          :     114
REMARK   3   BIN FREE R VALUE                    :   0.197
REMARK   3
REMARK   3  NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
REMARK   3   ALL ATOMS                 :     1940
REMARK   3
REMARK   3  B VALUES.
REMARK   3   FROM WILSON PLOT           (A**2) : NULL
REMARK   3   MEAN B VALUE      (OVERALL, A**2) :  13.666
REMARK   3   OVERALL ANISOTROPIC B VALUE.
REMARK   3    B11 (A**2) :    -0.02
REMARK   3    B22 (A**2) :    -0.02
REMARK   3    B33 (A**2) :     0.03
REMARK   3    B12 (A**2) :     0.00
REMARK   3    B13 (A**2) :     0.00
REMARK   3    B23 (A**2) :     0.00
REMARK   3
REMARK   3  ESTIMATED OVERALL COORDINATE ERROR.
REMARK   3   ESU BASED ON R VALUE                                (A):   0.092
REMARK   3   ESU BASED ON FREE R VALUE                           (A):   0.092
REMARK   3   ESU BASED ON MAXIMUM LIKELIHOOD                     (A):   0.075
REMARK   3   ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2):   2.208
REMARK   3
REMARK   3 CORRELATION COEFFICIENTS.
REMARK   3   CORRELATION COEFFICIENT FO-FC      :   0.947
REMARK   3   CORRELATION COEFFICIENT FO-FC FREE :   0.933
REMARK   3
REMARK   3  RMS DEVIATIONS FROM IDEAL VALUES        COUNT    RMS    WEIGHT
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REMARK   3   BOND LENGTHS REFINED ATOMS        (A):  1830 ; 0.010 ; 0.022
REMARK   3   BOND LENGTHS OTHERS               (A):  1593 ; 0.001 ; 0.020
REMARK   3   BOND ANGLES REFINED ATOMS   (DEGREES):  2490 ; 1.466 ; 1.941
REMARK   3   BOND ANGLES OTHERS          (DEGREES):  3724 ; 0.921 ; 3.000
REMARK   3   TORSION ANGLES, PERIOD 1    (DEGREES):   224 ; 4.899 ; 3.000
REMARK   3   TORSION ANGLES, PERIOD 3    (DEGREES):   311 ;16.844 ;15.000
REMARK   3   CHIRAL-CENTER RESTRAINTS       (A**3):   280 ; 0.231 ; 0.200
REMARK   3   GENERAL PLANES REFINED ATOMS      (A):  2026 ; 0.006 ; 0.020
REMARK   3   GENERAL PLANES OTHERS             (A):   374 ; 0.003 ; 0.020
REMARK   3   NON-BONDED CONTACTS REFINED ATOMS (A):   327 ; 0.271 ; 0.300
REMARK   3   NON-BONDED CONTACTS OTHERS        (A):  1447 ; 0.212 ; 0.300
REMARK   3   H-BOND (X...Y) REFINED ATOMS      (A):   131 ; 0.131 ; 0.500
REMARK   3   SYMMETRY VDW REFINED ATOMS        (A):     8 ; 0.310 ; 0.300
REMARK   3   SYMMETRY VDW OTHERS               (A):    17 ; 0.291 ; 0.300
REMARK   3   SYMMETRY H-BOND REFINED ATOMS     (A):    14 ; 0.144 ; 0.500
REMARK   3
REMARK   3  ISOTROPIC THERMAL FACTOR RESTRAINTS.      COUNT   RMS    WEIGHT
REMARK   3   MAIN-CHAIN BOND REFINED ATOMS  (A**2):  1124 ; 0.898 ; 1.500
REMARK   3   MAIN-CHAIN ANGLE REFINED ATOMS (A**2):  1827 ; 1.603 ; 2.000
REMARK   3   SIDE-CHAIN BOND REFINED ATOMS  (A**2):   706 ; 2.292 ; 3.000
REMARK   3   SIDE-CHAIN ANGLE REFINED ATOMS (A**2):   663 ; 3.839 ; 4.500
REMARK   3
REMARK   3  NCS RESTRAINTS STATISTICS
REMARK   3   NUMBER OF NCS GROUPS : NULL
REMARK   3
REMARK   3
REMARK   3  TLS DETAILS
REMARK   3   NUMBER OF TLS GROUPS  :    2
REMARK   3
REMARK   3   TLS GROUP :     1
REMARK   3    NUMBER OF COMPONENTS GROUP :    1
REMARK   3    COMPONENTS        C SSSEQI   TO  C SSSEQI
REMARK   3    RESIDUE RANGE :   A     1        A   113
REMARK   3    ORIGIN FOR THE GROUP (A):  31.8380  34.4130  15.9540
REMARK   3    T TENSOR
REMARK   3      T11:   0.0826 T22:   0.0528
REMARK   3      T33:   0.0022 T12:   0.0085
REMARK   3      T13:   0.0118 T23:   0.0066
REMARK   3    L TENSOR
REMARK   3      L11:   0.3236 L22:   1.6346
REMARK   3      L33:   0.0319 L12:  -0.4538
REMARK   3      L13:  -0.1060 L23:  -0.1134
REMARK   3    S TENSOR
REMARK   3      S11:   0.0668 S12:   0.0317 S13:   0.0266
REMARK   3      S21:  -0.0158 S22:  -0.0508 S23:  -0.0656
REMARK   3      S31:  -0.0111 S32:   0.0027 S33:  -0.0160
REMARK   3
REMARK   3   TLS GROUP :     2
REMARK   3    NUMBER OF COMPONENTS GROUP :    1
REMARK   3    COMPONENTS        C SSSEQI   TO  C SSSEQI
REMARK   3    RESIDUE RANGE :   B     1        B   112
REMARK   3    ORIGIN FOR THE GROUP (A):  33.7580   2.5150  18.4210
REMARK   3    T TENSOR
REMARK   3      T11:   0.0638 T22:   0.0608
REMARK   3      T33:   0.0227 T12:   0.0019
REMARK   3      T13:  -0.0064 T23:  -0.0055
REMARK   3    L TENSOR
REMARK   3      L11:   0.0923 L22:   0.6926
REMARK   3      L33:   0.1427 L12:  -0.1092
REMARK   3      L13:  -0.1135 L23:  -0.0160
REMARK   3    S TENSOR
REMARK   3      S11:   0.0096 S12:   0.0276 S13:  -0.0212
REMARK   3      S21:  -0.0046 S22:  -0.0327 S23:   0.0279
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REMARK   3      S31:  -0.0061 S32:  -0.0095 S33:   0.0231
REMARK   3
REMARK   3
REMARK   3  BULK SOLVENT MODELLING.
REMARK   3   METHOD USED : BABINET MODEL WITH MASK
REMARK   3   PARAMETERS FOR MASK CALCULATION
REMARK   3   VDW PROBE RADIUS   :   1.40
REMARK   3   ION PROBE RADIUS   :   0.80
REMARK   3   SHRINKAGE RADIUS   :   0.80
REMARK   3
REMARK   3  OTHER REFINEMENT REMARKS:
REMARK   3  HYDROGENS HAVE BEEN ADDED IN THE RIDING POSITIONS
REMARK   3
CISPEP   1 THR A   28    PRO A   29                    0.00
CISPEP   2 THR B   28    PRO B   29                    0.00
CRYST1   97.118   97.118   61.413  90.00  90.00  90.00 P 43 21 2
SCALE1      0.010297  0.000000  0.000000        0.00000
SCALE2      0.000000  0.010297  0.000000        0.00000
SCALE3      0.000000  0.000000  0.016283        0.00000
ATOM      1  O   ACE A   0      39.758  17.815   6.621  1.00 32.04           O
ATOM      2  C   ACE A   0      38.470  17.959   6.297  1.00 30.44           C
ATOM      3  CA  ACE A   0      37.841  19.332   5.940  1.00 30.13           C
ATOM      4  N   SER A   1      37.877  16.775   5.643  1.00 19.18           N
ATOM      6  CA  SER A   1      36.408  16.741   5.468  1.00 17.19           C
ATOM      8  CB  SER A   1      35.991  15.421   4.841  1.00 17.15           C
ATOM     11  OG  SER A   1      36.194  14.363   5.768  1.00 16.56           O
ATOM     13  C   SER A   1      35.748  16.842   6.834  1.00 16.94           C
ATOM     14  O   SER A   1      36.412  16.630   7.854  1.00 16.93           O
ATOM     17  N   ALA A   2      34.500  17.297   6.850  1.00 17.11           N
ATOM     19  CA  ALA A   2      33.637  17.247   8.031  1.00 16.12           C
ATOM     21  CB  ALA A   2      32.200  17.465   7.619  1.00 16.40           C
ATOM     25  C   ALA A   2      33.762  15.907   8.757  1.00 15.10           C
ATOM     26  O   ALA A   2      33.901  15.848   9.975  1.00 13.93           O
ATOM     27  N   THR A   3      33.680  14.823   8.009  1.00 14.66           N
ATOM     29  CA  THR A   3      33.773  13.515   8.630  1.00 13.12           C
ATOM     31  CB  THR A   3      33.497  12.440   7.599  1.00 13.38           C
ATOM     33  OG1 THR A   3      32.154  12.599   7.122  1.00 13.50           O
ATOM     35  CG2 THR A   3      33.517  11.067   8.238  1.00 14.13           C
ATOM     39  C   THR A   3      35.111  13.272   9.307  1.00 12.51           C
ATOM     40  O   THR A   3      35.141  12.780  10.440  1.00 10.83           O
ATOM     41  N   SER A   4      36.216  13.578   8.632  1.00 11.39           N
ATOM     43  CA  SER A   4      37.538  13.356   9.244  1.00 12.60           C
ATOM     45  CB  SER A   4      38.694  13.609   8.266  1.00 13.31           C
ATOM     48  OG  SER A   4      38.566  14.874   7.668  1.00 19.57           O
ATOM     50  C   SER A   4      37.726  14.223  10.471  1.00 11.69           C
ATOM     51  O   SER A   4      38.223  13.765  11.484  1.00 10.87           O
ATOM     52  N   LEU A   5      37.331  15.484  10.379  1.00 11.95           N
ATOM     54  CA  LEU A   5      37.478  16.382  11.515  1.00 11.00           C
ATOM     56  CB  LEU A   5      37.047  17.801  11.149  1.00 11.44           C
ATOM     59  CG  LEU A   5      37.928  18.509  10.117  1.00 13.46           C
ATOM     61  CD1 LEU A   5      37.267  19.790   9.651  1.00 15.05           C
ATOM     65  CD2 LEU A   5      39.270  18.807  10.731  1.00 15.52           C
ATOM     69  C   LEU A   5      36.658  15.900  12.698  1.00 10.25           C
ATOM     70  O   LEU A   5      37.114  15.947  13.852  1.00  9.79           O
ATOM     71  N   THR A   6      35.440  15.446  12.417  1.00  9.51           N
ATOM     73  CA  THR A   6      34.547  14.953  13.459  1.00  9.80           C
ATOM     75  CB  THR A   6      33.250  14.425  12.840  1.00  9.84           C
ATOM     77  OG1 THR A   6      32.454  15.510  12.319  1.00 10.30           O
ATOM     79  CG2 THR A   6      32.388  13.749  13.859  1.00  9.40           C
ATOM     83  C   THR A   6      35.186  13.816  14.236  1.00  9.72           C
ATOM     84  O   THR A   6      35.215  13.845  15.451  1.00  9.30           O
ATOM     85  N   PHE A   7      35.679  12.796  13.545  1.00  9.95           N
ATOM     87  CA  PHE A   7      36.185  11.642  14.278  1.00  8.92           C
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	ATOM	89	CB	PHE	A	7	35.993	10.367	13.490	1.00	8.90	C
	ATOM	92	CG	PHE	A	7	34.552	9.988	13.365	1.00	8.19	C
	ATOM	93	CD1	PHE	A	7	33.848	9.583	14.485	1.00	10.40	C
	ATOM	95	CE1	PHE	A	7	32.512	9.267	14.407	1.00	10.95	C
5	ATOM	97	CZ	PHE	A	7	31.848	9.370	13.217	1.00	11.35	C
	ATOM	99	CE2	PHE	A	7	32.532	9.791	12.080	1.00	10.55	C
	ATOM	101	CD2	PHE	A	7	33.872	10.127	12.165	1.00	10.65	C
	ATOM	103	C	PHE	A	7	37.603	11.819	14.812	1.00	9.58	C
	ATOM	104	O	PHE	A	7	37.970	11.203	15.811	1.00	9.17	O
10	ATOM	105	N	GLN	A	8	38.405	12.669	14.177	1.00	9.36	N
	ATOM	107	CA	GLN	A	8	39.683	12.999	14.778	1.00	10.36	C
	ATOM	109	CB	GLN	A	8	40.476	13.937	13.891	1.00	10.90	C
	ATOM	112	CG	GLN	A	8	41.097	13.322	12.692	1.00	14.14	C
	ATOM	115	CD	GLN	A	8	41.805	14.419	11.894	1.00	16.75	C
15	ATOM	116	OE1	GLN	A	8	41.409	14.742	10.787	1.00	21.77	O
	ATOM	117	NE2	GLN	A	8	42.799	15.056	12.517	1.00	20.28	N
	ATOM	120	C	GLN	A	8	39.409	13.716	16.116	1.00	10.53	C
	ATOM	121	O	GLN	A	8	40.049	13.416	17.118	1.00	10.95	O
	ATOM	122	N	LEU	A	9	38.457	14.654	16.122	1.00	9.95	N
20	ATOM	124	CA	LEU	A	9	38.145	15.413	17.332	1.00	9.62	C
	ATOM	126	CB	LEU	A	9	37.162	16.537	17.057	1.00	9.66	C
	ATOM	129	CG	LEU	A	9	36.767	17.375	18.278	1.00	9.80	C
	ATOM	131	CD1	LEU	A	9	37.974	18.098	18.862	1.00	10.08	C
	ATOM	135	CD2	LEU	A	9	35.701	18.397	17.886	1.00	12.75	C
25	ATOM	139	C	LEU	A	9	37.541	14.467	18.346	1.00	9.58	C
	ATOM	140	O	LEU	A	9	37.935	14.484	19.514	1.00	9.46	O
	ATOM	141	N	ALA	A	10	36.588	13.637	17.917	1.00	9.20	N
	ATOM	143	CA	ALA	A	10	35.952	12.701	18.856	1.00	9.03	C
	ATOM	145	CB	ALA	A	10	34.875	11.850	18.154	1.00	8.72	C
30	ATOM	149	C	ALA	A	10	36.949	11.802	19.605	1.00	8.50	C
	ATOM	150	O	ALA	A	10	36.855	11.615	20.825	1.00	8.50	O
	ATOM	151	N	TYR	A	11	37.918	11.242	18.899	1.00	9.18	N
	ATOM	153	CA	TYR	A	11	38.865	10.359	19.541	1.00	8.12	C
	ATOM	155	CB	TYR	A	11	39.716	9.664	18.491	1.00	8.30	C
35	ATOM	158	CG	TYR	A	11	40.642	8.638	19.075	1.00	7.61	C
	ATOM	159	CD1	TYR	A	11	40.156	7.495	19.699	1.00	9.07	C
	ATOM	161	CE1	TYR	A	11	41.008	6.560	20.229	1.00	10.41	C
	ATOM	163	CZ	TYR	A	11	42.359	6.768	20.170	1.00	13.73	C
	ATOM	164	OH	TYR	A	11	43.210	5.831	20.740	1.00	15.09	O
40	ATOM	166	CE2	TYR	A	11	42.868	7.898	19.571	1.00	10.94	C
	ATOM	168	CD2	TYR	A	11	42.014	8.827	19.027	1.00	10.00	C
	ATOM	170	C	TYR	A	11	39.752	11.139	20.530	1.00	8.66	C
	ATOM	171	O	TYR	A	11	40.158	10.596	21.550	1.00	8.96	O
	ATOM	172	N	LEU	A	12	40.012	12.412	20.245	1.00	8.35	N
45	ATOM	174	CA	LEU	A	12	40.899	13.238	21.081	1.00	9.68	C
	ATOM	176	CB	LEU	A	12	41.501	14.374	20.257	1.00	10.19	C
	ATOM	179	CG	LEU	A	12	42.469	13.943	19.152	1.00	15.33	C
	ATOM	181	CD1	LEU	A	12	43.187	15.145	18.549	1.00	18.28	C
	ATOM	185	CD2	LEU	A	12	43.464	12.905	19.653	1.00	18.55	C
50	ATOM	189	C	LEU	A	12	40.242	13.812	22.351	1.00	9.19	C
	ATOM	190	O	LEU	A	12	40.851	13.776	23.445	1.00	10.13	O
	ATOM	191	N	VAL	A	13	39.010	14.301	22.232	1.00	8.92	N
	ATOM	193	CA	VAL	A	13	38.357	14.969	23.368	1.00	8.52	C
	ATOM	195	CB	VAL	A	13	38.013	16.426	23.050	1.00	8.78	C
55	ATOM	197	CG1	VAL	A	13	39.251	17.141	22.537	1.00	10.74	C
	ATOM	201	CG2	VAL	A	13	36.864	16.560	22.057	1.00	9.49	C
	ATOM	205	C	VAL	A	13	37.131	14.252	23.904	1.00	8.44	C
	ATOM	206	O	VAL	A	13	36.592	14.631	24.947	1.00	8.60	O
	ATOM	207	N	LYS	A	14	36.709	13.218	23.178	1.00	8.48	N
60	ATOM	209	CA	LYS	A	14	35.583	12.339	23.536	1.00	8.98	C
	ATOM	211	CB	LYS	A	14	35.771	11.687	24.909	1.00	8.33	C
	ATOM	214	CG	LYS	A	14	37.127	11.029	25.118	1.00	7.66	C
	ATOM	217	CD	LYS	A	14	37.513	10.044	23.992	1.00	8.44	C

	ATOM	220	CE	LYS	A	14	38.818	9.318	24.229	1.00	7.68	C
	ATOM	223	NZ	LYS	A	14	39.160	8.416	23.087	1.00	7.55	N
	ATOM	227	C	LYS	A	14	34.187	12.932	23.465	1.00	10.23	C
	ATOM	228	O	LYS	A	14	33.306	12.332	22.864	1.00	9.28	O
5	ATOM	229	N	LYS	A	15	33.976	14.083	24.089	1.00	10.78	N
	ATOM	231	CA	LYS	A	15	32.636	14.648	24.202	1.00	12.04	C
	ATOM	233	CB	LYS	A	15	32.058	14.428	25.615	1.00	13.87	C
	ATOM	236	CG	LYS	A	15	30.626	14.970	25.767	1.00	18.29	C
	ATOM	239	CD	LYS	A	15	30.411	15.838	26.991	1.00	25.35	C
10	ATOM	242	CE	LYS	A	15	29.648	17.144	26.648	1.00	26.80	C
	ATOM	245	NZ	LYS	A	15	30.479	18.398	26.848	1.00	28.04	N
	ATOM	249	C	LYS	A	15	32.701	16.124	23.876	1.00	11.99	C
	ATOM	250	O	LYS	A	15	33.603	16.825	24.333	1.00	12.92	O
	ATOM	251	N	ILE	A	16	31.770	16.587	23.054	1.00	11.71	N
15	ATOM	253	CA	ILE	A	16	31.631	18.011	22.795	1.00	11.45	C
	ATOM	255	CB	ILE	A	16	32.644	18.502	21.769	1.00	12.21	C
	ATOM	257	CG1	ILE	A	16	32.966	19.980	22.019	1.00	12.61	C
	ATOM	260	CD1	ILE	A	16	34.167	20.459	21.239	1.00	16.67	C
	ATOM	264	CG2	ILE	A	16	32.154	18.226	20.357	1.00	12.62	C
20	ATOM	268	C	ILE	A	16	30.193	18.273	22.375	1.00	11.19	C
	ATOM	269	O	ILE	A	16	29.515	17.396	21.835	1.00	10.05	O
	ATOM	270	N	ASP	A	17	29.729	19.495	22.614	1.00	11.77	N
	ATOM	272	CA	ASP	A	17	28.357	19.861	22.315	1.00	11.36	C
	ATOM	274	CB	ASP	A	17	27.503	19.570	23.548	1.00	12.18	C
25	ATOM	277	CG	ASP	A	17	26.019	19.854	23.363	1.00	13.83	C
	ATOM	278	OD1	ASP	A	17	25.558	20.190	22.262	1.00	14.93	O
	ATOM	279	OD2	ASP	A	17	25.207	19.726	24.327	1.00	17.34	O
	ATOM	280	C	ASP	A	17	28.354	21.342	22.018	1.00	10.94	C
	ATOM	281	O	ASP	A	17	28.505	22.158	22.930	1.00	12.08	O
30	ATOM	282	N	PHE	A	18	28.220	21.709	20.754	1.00	9.97	N
	ATOM	284	CA	PHE	A	18	28.208	23.121	20.420	1.00	9.42	C
	ATOM	286	CB	PHE	A	18	29.621	23.630	20.070	1.00	9.10	C
	ATOM	289	CG	PHE	A	18	30.262	22.990	18.849	1.00	9.30	C
	ATOM	290	CD1	PHE	A	18	31.457	22.269	18.966	1.00	11.84	C
35	ATOM	292	CE1	PHE	A	18	32.069	21.704	17.850	1.00	11.09	C
	ATOM	294	CZ	PHE	A	18	31.520	21.860	16.619	1.00	10.73	C
	ATOM	296	CE2	PHE	A	18	30.335	22.573	16.470	1.00	11.19	C
	ATOM	298	CD2	PHE	A	18	29.725	23.157	17.586	1.00	8.90	C
	ATOM	300	C	PHE	A	18	27.226	23.431	19.299	1.00	9.78	C
40	ATOM	301	O	PHE	A	18	26.794	22.537	18.568	1.00	9.84	O
	ATOM	302	N	ASP	A	19	26.899	24.711	19.156	1.00	10.97	N
	ATOM	304	CA	ASP	A	19	26.059	25.169	18.060	1.00	10.37	C
	ATOM	306	CB	ASP	A	19	24.575	25.130	18.429	1.00	10.87	C
	ATOM	309	CG	ASP	A	19	23.674	25.452	17.267	1.00	11.55	C
45	ATOM	310	OD1	ASP	A	19	24.180	25.843	16.178	1.00	11.30	O
	ATOM	311	OD2	ASP	A	19	22.418	25.322	17.350	1.00	12.10	O
	ATOM	312	C	ASP	A	19	26.497	26.590	17.705	1.00	10.71	C
	ATOM	313	O	ASP	A	19	26.136	27.575	18.388	1.00	10.19	O
	ATOM	314	N	TYR	A	20	27.297	26.678	16.646	1.00	10.10	N
50	ATOM	316	CA	TYR	A	20	27.788	27.942	16.103	1.00	9.68	C
	ATOM	318	CB	TYR	A	20	29.308	27.879	15.911	1.00	9.82	C
	ATOM	321	CG	TYR	A	20	30.089	28.043	17.181	1.00	8.36	C
	ATOM	322	CD1	TYR	A	20	30.459	26.943	17.934	1.00	9.01	C
	ATOM	324	CE1	TYR	A	20	31.175	27.087	19.115	1.00	9.44	C
55	ATOM	326	CZ	TYR	A	20	31.514	28.335	19.546	1.00	10.02	C
	ATOM	327	OH	TYR	A	20	32.228	28.469	20.703	1.00	9.07	O
	ATOM	329	CE2	TYR	A	20	31.167	29.441	18.804	1.00	10.02	C
	ATOM	331	CD2	TYR	A	20	30.451	29.303	17.648	1.00	8.62	C
	ATOM	333	C	TYR	A	20	27.054	28.282	14.786	1.00	10.92	C
60	ATOM	334	O	TYR	A	20	27.600	28.930	13.878	1.00	11.60	O
	ATOM	335	N	THR	A	21	25.800	27.857	14.694	1.00	12.18	N
	ATOM	337	CA	THR	A	21	24.980	28.261	13.567	1.00	12.37	C
	ATOM	339	CB	THR	A	21	23.584	27.692	13.676	1.00	12.82	C

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ATOM    341  OG1 THR A  21      23.623  26.259  13.737  1.00 12.95           O
ATOM    343  CG2 THR A  21      22.832  27.997  12.401  1.00 13.70           C
ATOM    347  C   THR A  21      24.871  29.776  13.598  1.00 12.58           C
ATOM    348  O   THR A  21      24.445  30.332  14.595  1.00 12.57           O
ATOM    349  N   PRO A  22      25.259  30.460  12.528  1.00 12.83           N
ATOM    350  CA  PRO A  22      25.263  31.917  12.549  1.00 12.54           C
ATOM    352  CB  PRO A  22      26.214  32.276  11.409  1.00 12.71           C
ATOM    355  CG  PRO A  22      26.064  31.150  10.423  1.00 12.51           C
ATOM    358  CD  PRO A  22      25.773  29.925  11.259  1.00 12.22           C
ATOM    361  C   PRO A  22      23.890  32.509  12.337  1.00 12.87           C
ATOM    362  O   PRO A  22      23.281  32.302  11.282  1.00 14.33           O
ATOM    363  N   ASN A  23      23.405  33.202  13.363  1.00 12.69           N
ATOM    365  CA  ASN A  23      22.145  33.920  13.285  1.00 12.96           C
ATOM    367  CB  ASN A  23      21.290  33.568  14.497  1.00 13.22           C
ATOM    370  CG  ASN A  23      20.761  32.141  14.427  1.00 16.73           C
ATOM    371  OD1 ASN A  23      19.705  31.904  13.821  1.00 22.06           O
ATOM    372  ND2 ASN A  23      21.511  31.174  14.977  1.00 18.52           N
ATOM    375  C   ASN A  23      22.449  35.415  13.208  1.00 12.31           C
ATOM    376  O   ASN A  23      22.904  36.007  14.185  1.00 12.34           O
ATOM    377  N   TRP A  24      22.216  36.016  12.048  1.00 12.92           N
ATOM    379  CA  TRP A  24      22.554  37.408  11.814  1.00 12.37           C
ATOM    381  CB  TRP A  24      22.990  37.612  10.367  1.00 13.22           C
ATOM    384  CG  TRP A  24      24.130  36.740   9.944  1.00 12.12           C
ATOM    385  CD1 TRP A  24      24.039  35.556   9.279  1.00 11.86           C
ATOM    387  NE1 TRP A  24      25.292  35.046   9.042  1.00 13.92           N
ATOM    389  CE2 TRP A  24      26.230  35.904   9.547  1.00 11.19           C
ATOM    390  CD2 TRP A  24      25.536  36.989  10.123  1.00 10.96           C
ATOM    391  CE3 TRP A  24      26.276  38.003  10.726  1.00 11.62           C
ATOM    393  CZ3 TRP A  24      27.660  37.925  10.707  1.00 13.20           C
ATOM    395  CH2 TRP A  24      28.317  36.833  10.136  1.00 11.66           C
ATOM    397  CZ2 TRP A  24      27.619  35.814   9.545  1.00 10.81           C
ATOM    399  C   TRP A  24      21.343  38.268  12.120  1.00 12.73           C
ATOM    400  O   TRP A  24      20.282  38.076  11.532  1.00 13.03           O
ATOM    401  N   GLY A  25      21.488  39.222  13.029  1.00 12.00           N
ATOM    403  CA  GLY A  25      20.370  40.074  13.398  1.00 11.21           C
ATOM    406  C   GLY A  25      20.495  41.423  12.706  1.00 11.71           C
ATOM    407  O   GLY A  25      21.592  41.969  12.603  1.00 11.26           O
ATOM    408  N   ARG A  26      19.375  41.957  12.233  1.00 11.79           N
ATOM    410  CA  ARG A  26      19.388  43.192  11.486  1.00 12.49           C
ATOM    412  CB  ARG A  26      18.460  43.083  10.267  1.00 12.94           C
ATOM    415  CG  ARG A  26      18.999  42.137   9.202  1.00 16.01           C
ATOM    418  CD  ARG A  26      18.019  41.888   8.062  1.00 20.32           C
ATOM    421  NE  ARG A  26      18.565  41.043   6.998  1.00 24.78           N
ATOM    423  CZ  ARG A  26      19.426  41.460   6.071  1.00 25.04           C
ATOM    424  NH1 ARG A  26      19.860  40.607   5.149  1.00 29.16           N
ATOM    427  NH2 ARG A  26      19.863  42.715   6.057  1.00 19.47           N
ATOM    430  C   ARG A  26      19.010  44.365  12.357  1.00 12.60           C
ATOM    431  O   ARG A  26      18.369  44.206  13.398  1.00 12.88           O
ATOM    432  N   GLY A  27      19.411  45.549  11.917  1.00 12.77           N
ATOM    434  CA  GLY A  27      19.173  46.761  12.675  1.00 12.32           C
ATOM    437  C   GLY A  27      18.090  47.628  12.071  1.00 13.21           C
ATOM    438  O   GLY A  27      17.167  47.128  11.435  1.00 11.98           O
ATOM    439  N   THR A  28      18.203  48.928  12.316  1.00 14.26           N
ATOM    441  CA  THR A  28      17.261  49.925  11.819  1.00 14.60           C
ATOM    443  CB  THR A  28      16.523  50.576  13.006  1.00 13.94           C
ATOM    445  OG1 THR A  28      15.801  49.590  13.761  1.00 12.38           O
ATOM    447  CG2 THR A  28      15.460  51.569  12.517  1.00 14.06           C
ATOM    451  C   THR A  28      18.039  51.002  11.041  1.00 15.60           C
ATOM    452  O   THR A  28      18.823  51.756  11.636  1.00 15.37           O
ATOM    453  N   PRO A  29      17.874  51.082   9.718  1.00 17.62           N
ATOM    454  CA  PRO A  29      17.025  50.182   8.928  1.00 17.21           C
ATOM    456  CB  PRO A  29      16.956  50.887   7.570  1.00 17.70           C
ATOM    459  CG  PRO A  29      18.211  51.657   7.483  1.00 17.48           C
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	ATOM	462	CD	PRO	A	29	18.513	52.109	8.878	1.00	17.56	C
	ATOM	465	C	PRO	A	29	17.586	48.772	8.752	1.00	17.76	C
	ATOM	466	O	PRO	A	29	18.751	48.525	9.061	1.00	16.07	O
	ATOM	467	N	SER	A	30	16.742	47.873	8.242	1.00	18.81	N
5	ATOM	469	CA	SER	A	30	17.050	46.450	8.184	1.00	18.37	C
	ATOM	471	CB	SER	A	30	15.805	45.644	7.833	1.00	18.99	C
	ATOM	474	OG	SER	A	30	15.343	45.991	6.543	1.00	20.21	O
	ATOM	476	C	SER	A	30	18.169	46.068	7.246	1.00	17.73	C
	ATOM	477	O	SER	A	30	18.593	44.925	7.249	1.00	17.30	O
10	ATOM	478	N	SER	A	31	18.638	47.019	6.442	1.00	17.71	N
	ATOM	480	CA	SER	A	31	19.762	46.788	5.545	1.00	16.65	C
	ATOM	482	CB	SER	A	31	19.806	47.894	4.489	1.00	16.79	C
	ATOM	485	OG	SER	A	31	19.921	49.171	5.094	1.00	17.30	O
	ATOM	487	C	SER	A	31	21.098	46.709	6.297	1.00	16.11	C
15	ATOM	488	O	SER	A	31	22.127	46.365	5.704	1.00	15.78	O
	ATOM	489	N	TYR	A	32	21.086	47.032	7.597	1.00	14.64	N
	ATOM	491	CA	TYR	A	32	22.271	46.896	8.439	1.00	14.65	C
	ATOM	493	CB	TYR	A	32	22.375	48.046	9.422	1.00	14.98	C
	ATOM	496	CG	TYR	A	32	22.739	49.334	8.714	1.00	18.51	C
20	ATOM	497	CD1	TYR	A	32	24.066	49.674	8.496	1.00	21.23	C
	ATOM	499	CE1	TYR	A	32	24.407	50.838	7.829	1.00	23.56	C
	ATOM	501	CZ	TYR	A	32	23.413	51.669	7.369	1.00	24.54	C
	ATOM	502	OH	TYR	A	32	23.739	52.830	6.706	1.00	26.67	O
	ATOM	504	CE2	TYR	A	32	22.087	51.341	7.555	1.00	23.37	C
25	ATOM	506	CD2	TYR	A	32	21.758	50.174	8.225	1.00	21.68	C
	ATOM	508	C	TYR	A	32	22.237	45.591	9.229	1.00	13.62	C
	ATOM	509	O	TYR	A	32	21.188	45.198	9.725	1.00	13.16	O
	ATOM	510	N	ILE	A	33	23.380	44.911	9.286	1.00	13.61	N
	ATOM	512	CA	ILE	A	33	23.586	43.751	10.157	1.00	12.63	C
30	ATOM	514	CB	ILE	A	33	24.578	42.754	9.534	1.00	12.46	C
	ATOM	516	CG1	ILE	A	33	24.075	42.259	8.180	1.00	15.30	C
	ATOM	519	CD1	ILE	A	33	22.722	41.668	8.218	1.00	17.61	C
	ATOM	523	CG2	ILE	A	33	24.827	41.551	10.448	1.00	12.99	C
	ATOM	527	C	ILE	A	33	24.190	44.290	11.450	1.00	12.11	C
35	ATOM	528	O	ILE	A	33	25.296	44.828	11.452	1.00	11.53	O
	ATOM	529	N	ASP	A	34	23.471	44.131	12.551	1.00	12.39	N
	ATOM	531	CA	ASP	A	34	23.884	44.688	13.831	1.00	11.42	C
	ATOM	533	CB	ASP	A	34	22.658	45.171	14.607	1.00	11.00	C
	ATOM	536	CG	ASP	A	34	22.217	46.584	14.234	1.00	12.48	C
40	ATOM	537	OD1	ASP	A	34	22.658	47.096	13.183	1.00	13.04	O
	ATOM	538	OD2	ASP	A	34	21.399	47.223	14.951	1.00	13.34	O
	ATOM	539	C	ASP	A	34	24.580	43.675	14.723	1.00	10.32	C
	ATOM	540	O	ASP	A	34	25.317	44.056	15.621	1.00	10.05	O
	ATOM	541	N	ASN	A	35	24.321	42.394	14.504	1.00	10.16	N
45	ATOM	543	CA	ASN	A	35	24.851	41.393	15.433	1.00	9.67	C
	ATOM	545	CB	ASN	A	35	24.030	41.450	16.727	1.00	10.07	C
	ATOM	548	CG	ASN	A	35	22.554	41.273	16.471	1.00	10.85	C
	ATOM	549	OD1	ASN	A	35	22.107	40.167	16.176	1.00	12.45	O
	ATOM	550	ND2	ASN	A	35	21.783	42.374	16.550	1.00	9.57	N
50	ATOM	553	C	ASN	A	35	24.840	39.977	14.875	1.00	9.80	C
	ATOM	554	O	ASN	A	35	24.247	39.693	13.824	1.00	10.06	O
	ATOM	555	N	LEU	A	36	25.504	39.087	15.604	1.00	9.45	N
	ATOM	557	CA	LEU	A	36	25.636	37.689	15.262	1.00	9.51	C
	ATOM	559	CB	LEU	A	36	27.042	37.442	14.753	1.00	9.66	C
55	ATOM	562	CG	LEU	A	36	27.470	36.000	14.536	1.00	9.35	C
	ATOM	564	CD1	LEU	A	36	26.605	35.300	13.482	1.00	10.39	C
	ATOM	568	CD2	LEU	A	36	28.951	35.986	14.161	1.00	10.50	C
	ATOM	572	C	LEU	A	36	25.419	36.881	16.535	1.00	10.20	C
	ATOM	573	O	LEU	A	36	26.105	37.111	17.539	1.00	10.03	O
60	ATOM	574	N	THR	A	37	24.482	35.941	16.502	1.00	10.24	N
	ATOM	576	CA	THR	A	37	24.190	35.099	17.661	1.00	10.84	C
	ATOM	578	CB	THR	A	37	22.716	35.234	18.063	1.00	11.04	C
	ATOM	580	OG1	THR	A	37	22.440	36.591	18.421	1.00	12.32	O

	ATOM	582	CG2	THR	A	37	22.397	34.382	19.308	1.00	11.78	C
	ATOM	586	C	THR	A	37	24.484	33.647	17.365	1.00	10.36	C
	ATOM	587	O	THR	A	37	24.103	33.128	16.314	1.00	11.69	O
	ATOM	588	N	PHE	A	38	25.183	33.002	18.288	1.00	10.33	N
5	ATOM	590	CA	PHE	A	38	25.435	31.568	18.220	1.00	10.67	C
	ATOM	592	CB	PHE	A	38	26.892	31.285	18.520	1.00	10.97	C
	ATOM	595	CG	PHE	A	38	27.844	31.792	17.480	1.00	9.37	C
	ATOM	596	CD1	PHE	A	38	28.952	32.543	17.835	1.00	10.78	C
	ATOM	598	CE1	PHE	A	38	29.844	32.982	16.879	1.00	10.42	C
10	ATOM	600	CZ	PHE	A	38	29.659	32.667	15.590	1.00	12.03	C
	ATOM	602	CE2	PHE	A	38	28.559	31.898	15.215	1.00	10.68	C
	ATOM	604	CD2	PHE	A	38	27.660	31.476	16.146	1.00	10.68	C
	ATOM	606	C	PHE	A	38	24.595	30.912	19.303	1.00	11.13	C
	ATOM	607	O	PHE	A	38	24.678	31.328	20.444	1.00	11.20	O
15	ATOM	608	N	PRO	A	39	23.777	29.911	18.995	1.00	11.41	N
	ATOM	609	CA	PRO	A	39	22.920	29.317	20.033	1.00	11.03	C
	ATOM	611	CB	PRO	A	39	22.047	28.347	19.251	1.00	11.55	C
	ATOM	614	CG	PRO	A	39	22.138	28.792	17.827	1.00	11.41	C
	ATOM	617	CD	PRO	A	39	23.501	29.337	17.671	1.00	10.69	C
20	ATOM	620	C	PRO	A	39	23.593	28.585	21.186	1.00	10.78	C
	ATOM	621	O	PRO	A	39	23.007	28.537	22.272	1.00	11.13	O
	ATOM	622	N	LYS	A	40	24.756	27.986	20.961	1.00	10.03	N
	ATOM	624	CA	LYS	A	40	25.420	27.246	22.033	1.00	11.35	C
	ATOM	626	CB	LYS	A	40	24.930	25.808	22.100	1.00	11.81	C
25	ATOM	629	CG	LYS	A	40	25.329	25.153	23.413	1.00	15.47	C
	ATOM	632	CD	LYS	A	40	25.020	23.673	23.445	1.00	21.03	C
	ATOM	635	CE	LYS	A	40	25.654	23.024	24.665	1.00	26.85	C
	ATOM	638	NZ	LYS	A	40	24.928	23.362	25.917	1.00	35.22	N
	ATOM	642	C	LYS	A	40	26.939	27.297	21.877	1.00	11.13	C
30	ATOM	643	O	LYS	A	40	27.540	26.454	21.211	1.00	11.86	O
	ATOM	644	N	VAL	A	41	27.549	28.310	22.479	1.00	10.84	N
	ATOM	646	CA	VAL	A	41	28.995	28.462	22.410	1.00	10.68	C
	ATOM	648	CB	VAL	A	41	29.449	29.903	22.641	1.00	10.07	C
	ATOM	650	CG1	VAL	A	41	28.907	30.826	21.533	1.00	10.33	C
35	ATOM	654	CG2	VAL	A	41	29.040	30.419	24.007	1.00	10.45	C
	ATOM	658	C	VAL	A	41	29.690	27.564	23.425	1.00	11.85	C
	ATOM	659	O	VAL	A	41	29.093	27.111	24.425	1.00	12.38	O
	ATOM	660	N	LEU	A	42	30.957	27.305	23.165	1.00	13.03	N
	ATOM	662	CA	LEU	A	42	31.803	26.664	24.159	1.00	15.12	C
40	ATOM	664	CB	LEU	A	42	33.126	26.219	23.556	1.00	14.97	C
	ATOM	667	CG	LEU	A	42	32.873	25.139	22.491	1.00	15.42	C
	ATOM	669	CD1	LEU	A	42	34.128	24.763	21.705	1.00	16.85	C
	ATOM	673	CD2	LEU	A	42	32.303	23.917	23.125	1.00	17.26	C
	ATOM	677	C	LEU	A	42	32.012	27.709	25.245	1.00	17.87	C
45	ATOM	678	O	LEU	A	42	32.083	28.897	24.974	1.00	17.12	O
	ATOM	679	N	THR	A	43	32.171	27.279	26.476	1.00	21.75	N
	ATOM	681	CA	THR	A	43	32.188	28.272	27.549	1.00	24.61	C
	ATOM	683	CB	THR	A	43	30.761	28.365	28.043	1.00	24.82	C
	ATOM	685	OG1	THR	A	43	29.883	29.292	27.424	1.00	27.15	O
50	ATOM	687	CG2	THR	A	43	30.199	27.229	28.835	1.00	24.68	C
	ATOM	691	C	THR	A	43	33.197	27.863	28.620	1.00	26.54	C
	ATOM	692	O	THR	A	43	33.185	28.377	29.738	1.00	27.45	O
	ATOM	693	N	ASP	A	44	34.103	26.963	28.249	1.00	28.93	N
	ATOM	695	CA	ASP	A	44	35.103	26.469	29.179	1.00	29.14	C
55	ATOM	697	CB	ASP	A	44	35.855	25.271	28.602	1.00	28.74	C
	ATOM	700	CG	ASP	A	44	36.401	25.521	27.217	1.00	28.34	C
	ATOM	701	OD1	ASP	A	44	37.572	25.172	26.990	1.00	26.28	O
	ATOM	702	OD2	ASP	A	44	35.734	26.028	26.286	1.00	24.46	O
	ATOM	703	C	ASP	A	44	36.063	27.575	29.547	1.00	30.53	C
60	ATOM	704	O	ASP	A	44	36.513	27.663	30.699	1.00	30.19	O
	ATOM	705	N	LYS	A	45	36.372	28.422	28.568	1.00	31.95	N
	ATOM	707	CA	LYS	A	45	37.275	29.547	28.790	1.00	31.72	C
	ATOM	709	CB	LYS	A	45	38.701	29.244	28.320	1.00	31.90	C

	ATOM	712	CG	LYS	A	45	38.971	29.445	26.860	1.00	32.28	C
	ATOM	715	CD	LYS	A	45	39.201	28.149	26.171	1.00	33.73	C
	ATOM	718	CE	LYS	A	45	40.448	27.462	26.609	1.00	33.84	C
	ATOM	721	NZ	LYS	A	45	40.509	26.190	25.855	1.00	36.70	N
5	ATOM	725	C	LYS	A	45	36.715	30.803	28.140	1.00	31.38	C
	ATOM	726	O	LYS	A	45	35.679	30.756	27.482	1.00	31.01	O
	ATOM	727	N	LYS	A	46	37.399	31.925	28.352	1.00	31.71	N
	ATOM	729	CA	LYS	A	46	36.910	33.224	27.903	1.00	29.44	C
	ATOM	731	CB	LYS	A	46	37.338	34.330	28.875	1.00	30.08	C
10	ATOM	734	CG	LYS	A	46	38.819	34.397	29.144	1.00	32.08	C
	ATOM	737	CD	LYS	A	46	39.083	34.836	30.591	1.00	35.03	C
	ATOM	740	CE	LYS	A	46	40.571	34.941	30.910	1.00	36.79	C
	ATOM	743	NZ	LYS	A	46	40.827	34.716	32.367	1.00	37.85	N
	ATOM	747	C	LYS	A	46	37.335	33.551	26.488	1.00	26.70	C
15	ATOM	748	O	LYS	A	46	38.347	34.201	26.240	1.00	26.57	O
	ATOM	749	N	TYR	A	47	36.542	33.083	25.544	1.00	24.74	N
	ATOM	751	CA	TYR	A	47	36.802	33.387	24.144	1.00	20.86	C
	ATOM	753	CB	TYR	A	47	35.966	32.476	23.252	1.00	19.97	C
	ATOM	756	CG	TYR	A	47	36.251	31.026	23.482	1.00	17.82	C
20	ATOM	757	CD1	TYR	A	47	35.393	30.240	24.244	1.00	17.29	C
	ATOM	759	CE1	TYR	A	47	35.654	28.910	24.468	1.00	17.53	C
	ATOM	761	CZ	TYR	A	47	36.797	28.346	23.956	1.00	16.67	C
	ATOM	762	OH	TYR	A	47	37.076	27.005	24.174	1.00	20.90	O
	ATOM	764	CE2	TYR	A	47	37.670	29.109	23.205	1.00	17.26	C
25	ATOM	766	CD2	TYR	A	47	37.395	30.446	22.984	1.00	16.93	C
	ATOM	768	C	TYR	A	47	36.482	34.836	23.806	1.00	18.65	C
	ATOM	769	O	TYR	A	47	35.575	35.432	24.361	1.00	19.20	O
	ATOM	770	N	SER	A	48	37.229	35.388	22.863	1.00	16.14	N
	ATOM	772	CA	SER	A	48	36.957	36.716	22.329	1.00	14.79	C
30	ATOM	774	CB	SER	A	48	38.168	37.624	22.472	1.00	15.65	C
	ATOM	777	OG	SER	A	48	38.434	37.890	23.830	1.00	17.92	O
	ATOM	779	C	SER	A	48	36.638	36.586	20.852	1.00	12.87	C
	ATOM	780	O	SER	A	48	36.836	35.525	20.255	1.00	11.78	O
	ATOM	781	N	TYR	A	49	36.173	37.675	20.249	1.00	10.68	N
35	ATOM	783	CA	TYR	A	49	35.870	37.671	18.822	1.00	11.65	C
	ATOM	785	CB	TYR	A	49	34.362	37.641	18.580	1.00	11.29	C
	ATOM	788	CG	TYR	A	49	33.668	36.471	19.256	1.00	10.85	C
	ATOM	789	CD1	TYR	A	49	33.098	36.593	20.510	1.00	10.38	C
	ATOM	791	CE1	TYR	A	49	32.475	35.517	21.131	1.00	10.61	C
40	ATOM	793	CZ	TYR	A	49	32.404	34.310	20.480	1.00	12.03	C
	ATOM	794	OH	TYR	A	49	31.781	33.238	21.072	1.00	13.62	O
	ATOM	796	CE2	TYR	A	49	32.980	34.163	19.239	1.00	10.96	C
	ATOM	798	CD2	TYR	A	49	33.598	35.240	18.631	1.00	11.17	C
	ATOM	800	C	TYR	A	49	36.446	38.895	18.119	1.00	11.93	C
45	ATOM	801	O	TYR	A	49	36.259	40.028	18.564	1.00	12.07	O
	ATOM	802	N	ARG	A	50	37.122	38.649	17.004	1.00	13.41	N
	ATOM	804	CA	ARG	A	50	37.603	39.714	16.134	1.00	13.03	C
	ATOM	806	CB	ARG	A	50	38.983	39.376	15.561	1.00	13.84	C
	ATOM	809	CG	ARG	A	50	39.542	40.479	14.661	1.00	15.89	C
50	ATOM	812	CD	ARG	A	50	40.799	40.094	13.892	1.00	19.76	C
	ATOM	815	NE	ARG	A	50	41.825	39.658	14.809	1.00	22.85	N
	ATOM	817	CZ	ARG	A	50	42.474	40.468	15.643	1.00	29.33	C
	ATOM	818	NH1	ARG	A	50	43.391	39.966	16.456	1.00	35.65	N
	ATOM	821	NH2	ARG	A	50	42.224	41.779	15.666	1.00	30.97	N
55	ATOM	824	C	ARG	A	50	36.632	39.865	14.982	1.00	12.87	C
	ATOM	825	O	ARG	A	50	36.175	38.857	14.420	1.00	12.78	O
	ATOM	826	N	VAL	A	51	36.338	41.108	14.605	1.00	12.80	N
	ATOM	828	CA	VAL	A	51	35.419	41.393	13.506	1.00	13.04	C
	ATOM	830	CB	VAL	A	51	34.206	42.194	13.983	1.00	12.62	C
60	ATOM	832	CG1	VAL	A	51	33.343	42.630	12.809	1.00	12.65	C
	ATOM	836	CG2	VAL	A	51	33.389	41.356	14.936	1.00	12.04	C
	ATOM	840	C	VAL	A	51	36.167	42.170	12.438	1.00	13.60	C
	ATOM	841	O	VAL	A	51	36.851	43.153	12.738	1.00	13.27	O

	ATOM	842	N	VAL	A	52	36.074	41.685	11.206	1.00	14.90	N
	ATOM	844	CA	VAL	A	52	36.768	42.287	10.070	1.00	14.99	C
	ATOM	846	CB	VAL	A	52	37.834	41.307	9.534	1.00	15.36	C
	ATOM	848	CG1	VAL	A	52	38.577	41.908	8.360	1.00	15.94	C
5	ATOM	852	CG2	VAL	A	52	38.819	40.945	10.636	1.00	15.62	C
	ATOM	856	C	VAL	A	52	35.733	42.590	8.981	1.00	15.27	C
	ATOM	857	O	VAL	A	52	35.001	41.691	8.577	1.00	14.98	O
	ATOM	858	N	VAL	A	53	35.680	43.840	8.506	1.00	15.37	N
	ATOM	860	CA	VAL	A	53	34.663	44.255	7.542	1.00	16.36	C
10	ATOM	862	CB	VAL	A	53	33.805	45.395	8.090	1.00	16.50	C
	ATOM	864	CG1	VAL	A	53	32.827	45.905	7.043	1.00	16.92	C
	ATOM	868	CG2	VAL	A	53	33.037	44.923	9.314	1.00	16.68	C
	ATOM	872	C	VAL	A	53	35.366	44.712	6.284	1.00	17.90	C
	ATOM	873	O	VAL	A	53	36.121	45.670	6.321	1.00	17.86	O
15	ATOM	874	N	ASN	A	54	35.099	44.024	5.182	1.00	19.87	N
	ATOM	876	CA	ASN	A	54	35.764	44.316	3.916	1.00	20.93	C
	ATOM	878	CB	ASN	A	54	35.225	45.606	3.324	1.00	20.73	C
	ATOM	881	CG	ASN	A	54	33.946	45.408	2.504	1.00	20.64	C
	ATOM	882	OD1	ASN	A	54	33.395	46.382	1.976	1.00	22.37	O
20	ATOM	883	ND2	ASN	A	54	33.474	44.168	2.388	1.00	18.46	N
	ATOM	886	C	ASN	A	54	37.281	44.421	4.100	1.00	22.08	C
	ATOM	887	O	ASN	A	54	37.924	45.291	3.513	1.00	22.88	O
	ATOM	888	N	GLY	A	55	37.851	43.545	4.924	1.00	23.68	N
	ATOM	890	CA	GLY	A	55	39.288	43.532	5.134	1.00	22.59	C
25	ATOM	893	C	GLY	A	55	39.767	44.478	6.212	1.00	22.03	C
	ATOM	894	O	GLY	A	55	40.936	44.441	6.586	1.00	22.03	O
	ATOM	895	N	SER	A	56	38.883	45.332	6.712	1.00	21.22	N
	ATOM	897	CA	SER	A	56	39.268	46.257	7.764	1.00	20.83	C
	ATOM	899	CB	SER	A	56	38.434	47.521	7.666	1.00	21.16	C
30	ATOM	902	OG	SER	A	56	38.925	48.496	8.556	1.00	24.04	O
	ATOM	904	C	SER	A	56	39.068	45.628	9.138	1.00	19.96	C
	ATOM	905	O	SER	A	56	37.961	45.229	9.477	1.00	18.84	O
	ATOM	906	N	ASP	A	57	40.129	45.590	9.937	1.00	19.21	N
	ATOM	908	CA	ASP	A	57	40.100	44.953	11.252	1.00	19.05	C
35	ATOM	910	CB	ASP	A	57	41.547	44.599	11.610	1.00	19.38	C
	ATOM	913	CG	ASP	A	57	41.704	43.926	12.947	1.00	20.67	C
	ATOM	914	OD1	ASP	A	57	40.717	43.476	13.545	1.00	19.91	O
	ATOM	915	OD2	ASP	A	57	42.833	43.786	13.472	1.00	25.20	O
	ATOM	916	C	ASP	A	57	39.483	45.908	12.263	1.00	18.66	C
40	ATOM	917	O	ASP	A	57	40.031	46.992	12.524	1.00	17.62	O
	ATOM	918	N	LEU	A	58	38.337	45.517	12.823	1.00	18.14	N
	ATOM	920	CA	LEU	A	58	37.660	46.339	13.821	1.00	17.44	C
	ATOM	922	CB	LEU	A	58	36.140	46.283	13.638	1.00	17.54	C
	ATOM	925	CG	LEU	A	58	35.587	46.711	12.271	1.00	18.21	C
45	ATOM	927	CD1	LEU	A	58	34.067	46.915	12.314	1.00	18.79	C
	ATOM	931	CD2	LEU	A	58	36.271	47.970	11.777	1.00	20.33	C
	ATOM	935	C	LEU	A	58	38.058	45.955	15.248	1.00	17.13	C
	ATOM	936	O	LEU	A	58	37.539	46.510	16.221	1.00	17.54	O
	ATOM	937	N	GLY	A	59	38.978	45.010	15.381	1.00	16.87	N
50	ATOM	939	CA	GLY	A	59	39.503	44.667	16.686	1.00	16.43	C
	ATOM	942	C	GLY	A	59	38.781	43.524	17.361	1.00	16.14	C
	ATOM	943	O	GLY	A	59	37.953	42.845	16.768	1.00	13.91	O
	ATOM	944	N	VAL	A	60	39.070	43.377	18.641	1.00	16.63	N
	ATOM	946	CA	VAL	A	60	38.664	42.216	19.409	1.00	16.80	C
55	ATOM	948	CB	VAL	A	60	39.909	41.452	19.859	1.00	17.07	C
	ATOM	950	CG1	VAL	A	60	39.536	40.267	20.694	1.00	17.82	C
	ATOM	954	CG2	VAL	A	60	40.719	40.997	18.636	1.00	17.98	C
	ATOM	958	C	VAL	A	60	37.883	42.635	20.638	1.00	17.13	C
	ATOM	959	O	VAL	A	60	38.254	43.594	21.331	1.00	17.22	O
60	ATOM	960	N	GLU	A	61	36.806	41.913	20.913	1.00	16.81	N
	ATOM	962	CA	GLU	A	61	35.954	42.215	22.058	1.00	17.72	C
	ATOM	964	CB	GLU	A	61	34.759	43.060	21.623	1.00	18.26	C
	ATOM	967	CG	GLU	A	61	35.079	44.412	20.956	1.00	20.64	C

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ATOM    970  CD  GLU A  61      35.548  45.510  21.912  1.00 24.07           C
ATOM    971  OE1 GLU A  61      35.294  45.417  23.142  1.00 25.24           O
ATOM    972  OE2 GLU A  61      36.174  46.484  21.416  1.00 24.17           O
ATOM    973  C   GLU A  61      35.477  40.897  22.667  1.00 18.17           C
ATOM    974  O   GLU A  61      35.387  39.870  21.972  1.00 15.50           O
ATOM    975  N   SER A  62      35.171  40.917  23.964  1.00 19.13           N
ATOM    977  CA  SER A  62      34.710  39.697  24.634  1.00 20.18           C
ATOM    979  CB  SER A  62      35.838  39.109  25.479  1.00 20.45           C
ATOM    982  OG  SER A  62      36.229  40.016  26.499  1.00 21.81           O
ATOM    984  C   SER A  62      33.488  39.884  25.537  1.00 20.19           C
ATOM    985  O   SER A  62      32.920  38.912  26.038  1.00 20.33           O
ATOM    986  N   ASN A  63      33.073  41.120  25.735  1.00 20.95           N
ATOM    988  CA  ASN A  63      32.043  41.388  26.729  1.00 21.35           C
ATOM    990  CB  ASN A  63      32.310  42.725  27.418  1.00 22.62           C
ATOM    993  CG  ASN A  63      31.947  43.893  26.582  1.00 26.10           C
ATOM    994  OD1 ASN A  63      31.697  44.985  27.106  1.00 33.95           O
ATOM    995  ND2 ASN A  63      31.936  43.704  25.268  1.00 38.69           N
ATOM    998  C   ASN A  63      30.655  41.248  26.135  1.00 19.66           C
ATOM    999  O   ASN A  63      29.954  42.221  25.801  1.00 20.53           O
ATOM   1000  N   PHE A  64      30.318  39.982  25.925  1.00 17.32           N
ATOM   1002  CA  PHE A  64      29.024  39.592  25.437  1.00 15.47           C
ATOM   1004  CB  PHE A  64      29.125  39.076  23.995  1.00 14.91           C
ATOM   1007  CG  PHE A  64      29.885  40.014  23.077  1.00 13.87           C
ATOM   1008  CD1 PHE A  64      29.388  41.270  22.792  1.00 14.13           C
ATOM   1010  CE1 PHE A  64      30.091  42.136  21.982  1.00 14.78           C
ATOM   1012  CZ  PHE A  64      31.299  41.748  21.441  1.00 12.67           C
ATOM   1014  CE2 PHE A  64      31.808  40.511  21.723  1.00 13.44           C
ATOM   1016  CD2 PHE A  64      31.108  39.644  22.529  1.00 13.11           C
ATOM   1018  C   PHE A  64      28.561  38.496  26.376  1.00 14.27           C
ATOM   1019  O   PHE A  64      29.242  37.490  26.585  1.00 12.73           O
ATOM   1020  N   ALA A  65      27.382  38.708  26.928  1.00 14.30           N
ATOM   1022  CA  ALA A  65      26.782  37.806  27.875  1.00 14.41           C
ATOM   1024  CB  ALA A  65      25.441  38.380  28.300  1.00 14.45           C
ATOM   1028  C   ALA A  65      26.581  36.424  27.282  1.00 14.66           C
ATOM   1029  O   ALA A  65      26.244  36.311  26.098  1.00 15.01           O
ATOM   1030  N   VAL A  66      26.796  35.389  28.086  1.00 15.36           N
ATOM   1032  CA  VAL A  66      26.427  34.049  27.683  1.00 15.61           C
ATOM   1034  CB  VAL A  66      27.484  32.994  27.972  1.00 15.66           C
ATOM   1036  CG1 VAL A  66      26.958  31.609  27.592  1.00 17.06           C
ATOM   1040  CG2 VAL A  66      28.754  33.275  27.215  1.00 16.50           C
ATOM   1044  C   VAL A  66      25.158  33.766  28.476  1.00 15.70           C
ATOM   1045  O   VAL A  66      25.098  33.936  29.705  1.00 17.12           O
ATOM   1046  N   THR A  67      24.115  33.379  27.777  1.00 15.06           N
ATOM   1048  CA  THR A  67      22.854  33.106  28.439  1.00 15.98           C
ATOM   1050  CB  THR A  67      21.681  33.345  27.491  1.00 15.54           C
ATOM   1052  OG1 THR A  67      21.794  32.535  26.311  1.00 14.59           O
ATOM   1054  CG2 THR A  67      21.718  34.774  26.958  1.00 15.99           C
ATOM   1058  C   THR A  67      22.910  31.687  29.016  1.00 16.78           C
ATOM   1059  O   THR A  67      23.742  30.885  28.620  1.00 16.89           O
ATOM   1060  N   PRO A  68      22.150  31.418  30.062  1.00 18.84           N
ATOM   1061  CA  PRO A  68      22.093  30.058  30.617  1.00 19.21           C
ATOM   1063  CB  PRO A  68      20.997  30.168  31.683  1.00 19.65           C
ATOM   1066  CG  PRO A  68      21.101  31.602  32.125  1.00 18.30           C
ATOM   1069  CD  PRO A  68      21.436  32.395  30.897  1.00 18.90           C
ATOM   1072  C   PRO A  68      21.826  28.955  29.582  1.00 20.21           C
ATOM   1073  O   PRO A  68      22.274  27.827  29.790  1.00 19.65           O
ATOM   1074  N   SER A  69      21.145  29.278  28.485  1.00 22.19           N
ATOM   1076  CA  SER A  69      20.918  28.325  27.390  1.00 21.01           C
ATOM   1078  CB  SER A  69      19.822  28.847  26.463  1.00 21.48           C
ATOM   1081  OG  SER A  69      20.198  30.084  25.869  1.00 21.75           O
ATOM   1083  C   SER A  69      22.189  28.062  26.582  1.00 20.29           C
ATOM   1084  O   SER A  69      22.276  27.090  25.825  1.00 20.29           O
ATOM   1085  N   GLY A  70      23.185  28.926  26.742  1.00 18.45           N
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ATOM	1087	CA	GLY	A	70	24.455	28.736	26.089	1.00	16.37	C
ATOM	1090	C	GLY	A	70	24.635	29.701	24.941	1.00	14.59	C
ATOM	1091	O	GLY	A	70	25.655	29.678	24.275	1.00	14.71	O
ATOM	1092	N	GLY	A	71	23.655	30.564	24.707	1.00	12.55	N
ATOM	1094	CA	GLY	A	71	23.758	31.485	23.587	1.00	11.79	C
ATOM	1097	C	GLY	A	71	24.646	32.689	23.872	1.00	11.04	C
ATOM	1098	O	GLY	A	71	24.827	33.109	25.024	1.00	11.27	O
ATOM	1099	N	GLN	A	72	25.209	33.247	22.807	1.00	10.97	N
ATOM	1101	CA	GLN	A	72	26.016	34.462	22.914	1.00	10.54	C
ATOM	1103	CB	GLN	A	72	27.497	34.125	23.115	1.00	10.98	C
ATOM	1106	CG	GLN	A	72	28.414	35.293	23.430	1.00	12.20	C
ATOM	1109	CD	GLN	A	72	29.834	34.862	23.853	1.00	15.65	C
ATOM	1110	OE1	GLN	A	72	30.449	35.487	24.742	1.00	17.66	O
ATOM	1111	NE2	GLN	A	72	30.354	33.820	23.222	1.00	10.29	N
ATOM	1114	C	GLN	A	72	25.807	35.312	21.675	1.00	11.06	C
ATOM	1115	O	GLN	A	72	25.877	34.821	20.533	1.00	11.31	O
ATOM	1116	N	THR	A	73	25.535	36.589	21.904	1.00	10.95	N
ATOM	1118	CA	THR	A	73	25.337	37.526	20.830	1.00	9.80	C
ATOM	1120	CB	THR	A	73	24.021	38.290	21.035	1.00	10.73	C
ATOM	1122	OG1	THR	A	73	22.912	37.385	21.013	1.00	11.04	O
ATOM	1124	CG2	THR	A	73	23.786	39.270	19.891	1.00	10.78	C
ATOM	1128	C	THR	A	73	26.475	38.540	20.782	1.00	9.92	C
ATOM	1129	O	THR	A	73	26.722	39.283	21.745	1.00	10.19	O
ATOM	1130	N	ILE	A	74	27.161	38.554	19.643	1.00	9.37	N
ATOM	1132	CA	ILE	A	74	28.232	39.493	19.364	1.00	10.19	C
ATOM	1134	CB	ILE	A	74	29.235	38.855	18.371	1.00	10.48	C
ATOM	1136	CG1	ILE	A	74	29.843	37.581	18.972	1.00	12.71	C
ATOM	1139	CD1	ILE	A	74	30.471	36.666	17.946	1.00	16.05	C
ATOM	1143	CG2	ILE	A	74	30.296	39.860	17.986	1.00	10.70	C
ATOM	1147	C	ILE	A	74	27.609	40.733	18.756	1.00	10.18	C
ATOM	1148	O	ILE	A	74	27.052	40.677	17.660	1.00	11.08	O
ATOM	1149	N	ASN	A	75	27.674	41.851	19.489	1.00	9.17	N
ATOM	1151	CA	ASN	A	75	27.079	43.102	19.040	1.00	9.50	C
ATOM	1153	CB	ASN	A	75	26.600	43.849	20.274	1.00	9.51	C
ATOM	1156	CG	ASN	A	75	25.994	45.177	19.950	1.00	10.45	C
ATOM	1157	OD1	ASN	A	75	25.558	45.424	18.827	1.00	9.62	O
ATOM	1158	ND2	ASN	A	75	25.931	46.046	20.959	1.00	12.30	N
ATOM	1161	C	ASN	A	75	28.050	43.975	18.248	1.00	9.58	C
ATOM	1162	O	ASN	A	75	28.992	44.543	18.807	1.00	10.09	O
ATOM	1163	N	PHE	A	76	27.817	44.088	16.945	1.00	10.23	N
ATOM	1165	CA	PHE	A	76	28.751	44.809	16.087	1.00	10.31	C
ATOM	1167	CB	PHE	A	76	28.464	44.552	14.610	1.00	10.82	C
ATOM	1170	CG	PHE	A	76	28.596	43.096	14.199	1.00	11.07	C
ATOM	1171	CD1	PHE	A	76	29.568	42.277	14.737	1.00	13.37	C
ATOM	1173	CE1	PHE	A	76	29.681	40.936	14.328	1.00	10.49	C
ATOM	1175	CZ	PHE	A	76	28.820	40.441	13.411	1.00	10.42	C
ATOM	1177	CE2	PHE	A	76	27.856	41.259	12.865	1.00	11.96	C
ATOM	1179	CD2	PHE	A	76	27.746	42.568	13.258	1.00	12.00	C
ATOM	1181	C	PHE	A	76	28.780	46.301	16.409	1.00	10.53	C
ATOM	1182	O	PHE	A	76	29.743	46.978	16.059	1.00	10.34	O
ATOM	1183	N	LEU	A	77	27.746	46.826	17.073	1.00	10.19	N
ATOM	1185	CA	LEU	A	77	27.754	48.242	17.446	1.00	11.27	C
ATOM	1187	CB	LEU	A	77	26.443	48.652	18.120	1.00	11.26	C
ATOM	1190	CG	LEU	A	77	25.267	48.913	17.154	1.00	12.41	C
ATOM	1192	CD1	LEU	A	77	24.989	47.774	16.232	1.00	12.55	C
ATOM	1196	CD2	LEU	A	77	23.977	49.223	17.911	1.00	13.90	C
ATOM	1200	C	LEU	A	77	28.933	48.577	18.368	1.00	12.07	C
ATOM	1201	O	LEU	A	77	29.399	49.717	18.371	1.00	13.10	O
ATOM	1202	N	GLN	A	78	29.416	47.580	19.112	1.00	12.87	N
ATOM	1204	CA	GLN	A	78	30.562	47.741	20.011	1.00	13.30	C
ATOM	1206	CB	GLN	A	78	30.588	46.602	21.048	1.00	13.42	C
ATOM	1209	CG	GLN	A	78	29.408	46.690	22.022	1.00	14.19	C
ATOM	1212	CD	GLN	A	78	29.251	45.560	23.009	1.00	17.92	C

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ATOM   1213  OE1 GLN A  78      28.141  45.045  23.165  1.00 20.68           O
ATOM   1214  NE2 GLN A  78      30.316  45.212  23.726  1.00 21.02           N
ATOM   1217  C   GLN A  78      31.892  47.837  19.231  1.00 14.14           C
ATOM   1218  O   GLN A  78      32.905  48.303  19.774  1.00 16.34           O
ATOM   1219  N   TYR A  79      31.896  47.398  17.979  1.00 13.44           N
ATOM   1221  CA  TYR A  79      33.101  47.443  17.143  1.00 13.76           C
ATOM   1223  CB  TYR A  79      33.220  46.165  16.311  1.00 13.39           C
ATOM   1226  CG  TYR A  79      33.402  44.856  17.041  1.00 12.44           C
ATOM   1227  CD1 TYR A  79      34.649  44.249  17.117  1.00 11.93           C
ATOM   1229  CE1 TYR A  79      34.816  43.040  17.755  1.00 11.71           C
ATOM   1231  CZ  TYR A  79      33.712  42.414  18.310  1.00 12.16           C
ATOM   1232  OH  TYR A  79      33.873  41.215  18.929  1.00 13.43           O
ATOM   1234  CE2 TYR A  79      32.463  43.002  18.239  1.00 10.82           C
ATOM   1236  CD2 TYR A  79      32.319  44.204  17.607  1.00 11.32           C
ATOM   1238  C   TYR A  79      33.092  48.571  16.111  1.00 14.75           C
ATOM   1239  O   TYR A  79      34.154  48.964  15.600  1.00 14.73           O
ATOM   1240  N   ASN A  80      31.899  49.072  15.786  1.00 15.93           N
ATOM   1242  CA  ASN A  80      31.702  49.928  14.614  1.00 15.93           C
ATOM   1244  CB  ASN A  80      30.814  49.164  13.619  1.00 15.91           C
ATOM   1247  CG  ASN A  80      30.884  49.703  12.203  1.00 17.60           C
ATOM   1248  OD1 ASN A  80      29.863  49.757  11.507  1.00 22.84           O
ATOM   1249  ND2 ASN A  80      32.067  50.104  11.767  1.00 16.39           N
ATOM   1252  C   ASN A  80      31.101  51.293  14.941  1.00 16.30           C
ATOM   1253  O   ASN A  80      30.238  51.789  14.220  1.00 16.27           O
ATOM   1254  N   LYS A  81      31.559  51.881  16.042  1.00 16.66           N
ATOM   1256  CA  LYS A  81      31.166  53.238  16.448  1.00 17.75           C
ATOM   1258  CB  LYS A  81      31.753  54.274  15.485  1.00 18.72           C
ATOM   1261  CG  LYS A  81      33.275  54.164  15.279  1.00 22.17           C
ATOM   1264  CD  LYS A  81      34.098  53.637  16.494  1.00 28.26           C
ATOM   1267  CE  LYS A  81      33.999  54.432  17.816  1.00 31.88           C
ATOM   1270  NZ  LYS A  81      34.916  53.895  18.908  1.00 34.98           N
ATOM   1274  C   LYS A  81      29.660  53.445  16.590  1.00 17.22           C
ATOM   1275  O   LYS A  81      29.139  54.507  16.230  1.00 16.93           O
ATOM   1276  N   GLY A  82      28.969  52.429  17.115  1.00 16.00           N
ATOM   1278  CA  GLY A  82      27.546  52.523  17.393  1.00 16.21           C
ATOM   1281  C   GLY A  82      26.612  52.249  16.232  1.00 16.87           C
ATOM   1282  O   GLY A  82      25.406  52.461  16.362  1.00 16.37           O
ATOM   1283  N   TYR A  83      27.152  51.772  15.111  1.00 16.99           N
ATOM   1285  CA  TYR A  83      26.342  51.425  13.958  1.00 17.84           C
ATOM   1287  CB  TYR A  83      26.751  52.240  12.735  1.00 18.95           C
ATOM   1290  CG  TYR A  83      26.375  53.678  12.816  1.00 24.21           C
ATOM   1291  CD1 TYR A  83      25.138  54.103  12.373  1.00 29.21           C
ATOM   1293  CE1 TYR A  83      24.775  55.428  12.440  1.00 31.15           C
ATOM   1295  CZ  TYR A  83      25.660  56.351  12.956  1.00 32.63           C
ATOM   1296  OH  TYR A  83      25.291  57.680  13.016  1.00 35.54           O
ATOM   1298  CE2 TYR A  83      26.906  55.954  13.404  1.00 31.21           C
ATOM   1300  CD2 TYR A  83      27.258  54.618  13.327  1.00 28.33           C
ATOM   1302  C   TYR A  83      26.576  49.990  13.590  1.00 16.45           C
ATOM   1303  O   TYR A  83      27.652  49.438  13.854  1.00 16.18           O
ATOM   1304  N   GLY A  84      25.570  49.372  12.982  1.00 14.45           N
ATOM   1306  CA  GLY A  84      25.761  48.049  12.430  1.00 14.93           C
ATOM   1309  C   GLY A  84      26.514  48.187  11.109  1.00 15.19           C
ATOM   1310  O   GLY A  84      27.001  49.273  10.757  1.00 15.50           O
ATOM   1311  N   VAL A  85      26.565  47.099  10.353  1.00 15.19           N
ATOM   1313  CA  VAL A  85      27.352  47.043   9.112  1.00 15.08           C
ATOM   1315  CB  VAL A  85      28.332  45.860   9.143  1.00 15.27           C
ATOM   1317  CG1 VAL A  85      29.157  45.794   7.872  1.00 15.21           C
ATOM   1321  CG2 VAL A  85      29.255  45.956  10.329  1.00 15.25           C
ATOM   1325  C   VAL A  85      26.438  46.873   7.911  1.00 15.53           C
ATOM   1326  O   VAL A  85      25.551  46.025   7.910  1.00 14.72           O
ATOM   1327  N   ALA A  86      26.648  47.673   6.875  1.00 15.80           N
ATOM   1329  CA  ALA A  86      25.802  47.548   5.698  1.00 16.04           C
ATOM   1331  CB  ALA A  86      26.250  48.488   4.631  1.00 16.32           C
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ATOM   1335  C   ALA A  86      25.861  46.128   5.186  1.00 15.57           C
ATOM   1336  O   ALA A  86      26.941  45.575   5.059  1.00 15.01           O
ATOM   1337  N   ASP A  87      24.708  45.558   4.842  1.00 16.64           N
ATOM   1339  CA  ASP A  87      24.658  44.160   4.402  1.00 16.37           C
ATOM   1341  CB  ASP A  87      23.253  43.550   4.495  1.00 16.92           C
ATOM   1344  CG  ASP A  87      22.293  44.088   3.472  1.00 17.15           C
ATOM   1345  OD1 ASP A  87      21.117  43.677   3.520  1.00 17.33           O
ATOM   1346  OD2 ASP A  87      22.615  44.920   2.605  1.00 17.89           O
ATOM   1347  C   ASP A  87      25.316  43.899   3.046  1.00 16.95           C
ATOM   1348  O   ASP A  87      25.392  42.753   2.623  1.00 16.19           O
ATOM   1349  N   THR A  88      25.812  44.949   2.398  1.00 18.29           N
ATOM   1351  CA  THR A  88      26.566  44.803   1.146  1.00 17.50           C
ATOM   1353  CB  THR A  88      26.427  46.084   0.327  1.00 17.73           C
ATOM   1355  OG1 THR A  88      26.702  47.225   1.150  1.00 16.82           O
ATOM   1357  CG2 THR A  88      25.020  46.269  -0.109  1.00 18.14           C
ATOM   1361  C   THR A  88      28.052  44.563   1.361  1.00 17.57           C
ATOM   1362  O   THR A  88      28.820  44.404   0.409  1.00 16.74           O
ATOM   1363  N   LYS A  89      28.477  44.594   2.609  1.00 17.46           N
ATOM   1365  CA  LYS A  89      29.871  44.389   2.919  1.00 17.25           C
ATOM   1367  CB  LYS A  89      30.312  45.388   3.978  1.00 17.22           C
ATOM   1370  CG  LYS A  89      30.058  46.844   3.579  1.00 18.92           C
ATOM   1373  CD  LYS A  89      30.818  47.788   4.471  1.00 22.27           C
ATOM   1376  CE  LYS A  89      30.590  49.242   4.055  1.00 25.20           C
ATOM   1379  NZ  LYS A  89      31.208  50.160   5.042  1.00 29.86           N
ATOM   1383  C   LYS A  89      30.069  42.968   3.411  1.00 17.09           C
ATOM   1384  O   LYS A  89      29.122  42.311   3.818  1.00 16.84           O
ATOM   1385  N   THR A  90      31.300  42.493   3.343  1.00 17.10           N
ATOM   1387  CA  THR A  90      31.662  41.181   3.842  1.00 16.27           C
ATOM   1389  CB  THR A  90      32.860  40.644   3.086  1.00 17.13           C
ATOM   1391  OG1 THR A  90      32.533  40.515   1.704  1.00 15.79           O
ATOM   1393  CG2 THR A  90      33.199  39.226   3.543  1.00 17.35           C
ATOM   1397  C   THR A  90      32.068  41.322   5.296  1.00 15.60           C
ATOM   1398  O   THR A  90      32.930  42.137   5.613  1.00 14.66           O
ATOM   1399  N   ILE A  91      31.451  40.543   6.170  1.00 15.11           N
ATOM   1401  CA  ILE A  91      31.823  40.561   7.577  1.00 14.05           C
ATOM   1403  CB  ILE A  91      30.596  40.777   8.475  1.00 14.05           C
ATOM   1405  CG1 ILE A  91      29.771  41.971   7.995  1.00 13.77           C
ATOM   1408  CD1 ILE A  91      28.482  42.119   8.725  1.00 15.25           C
ATOM   1412  CG2 ILE A  91      31.039  40.924   9.949  1.00 13.82           C
ATOM   1416  C   ILE A  91      32.435  39.221   7.914  1.00 14.03           C
ATOM   1417  O   ILE A  91      31.795  38.191   7.702  1.00 14.85           O
ATOM   1418  N   GLN A  92      33.679  39.230   8.382  1.00 12.86           N
ATOM   1420  CA  GLN A  92      34.298  38.028   8.919  1.00 13.25           C
ATOM   1422  CB  GLN A  92      35.678  37.818   8.338  1.00 14.29           C
ATOM   1425  CG  GLN A  92      35.645  37.428   6.904  1.00 16.51           C
ATOM   1428  CD  GLN A  92      37.020  37.515   6.275  1.00 21.30           C
ATOM   1429  OE1 GLN A  92      37.536  36.517   5.775  1.00 25.59           O
ATOM   1430  NE2 GLN A  92      37.627  38.701   6.319  1.00 23.68           N
ATOM   1433  C   GLN A  92      34.443  38.120  10.423  1.00 12.74           C
ATOM   1434  O   GLN A  92      34.914  39.127  10.940  1.00 12.27           O
ATOM   1435  N   VAL A  93      34.072  37.051  11.115  1.00 11.90           N
ATOM   1437  CA  VAL A  93      34.217  36.985  12.564  1.00 11.73           C
ATOM   1439  CB  VAL A  93      32.865  36.841  13.257  1.00 11.43           C
ATOM   1441  CG1 VAL A  93      33.048  36.856  14.771  1.00 12.22           C
ATOM   1445  CG2 VAL A  93      31.925  37.956  12.809  1.00 12.10           C
ATOM   1449  C   VAL A  93      35.118  35.797  12.912  1.00 11.97           C
ATOM   1450  O   VAL A  93      34.953  34.707  12.379  1.00 11.23           O
ATOM   1451  N   PHE A  94      36.096  36.055  13.773  1.00 11.48           N
ATOM   1453  CA  PHE A  94      37.069  35.064  14.188  1.00 12.22           C
ATOM   1455  CB  PHE A  94      38.473  35.563  13.871  1.00 12.62           C
ATOM   1458  CG  PHE A  94      38.736  35.743  12.404  1.00 12.77           C
ATOM   1459  CD1 PHE A  94      38.345  36.900  11.763  1.00 14.64           C
ATOM   1461  CE1 PHE A  94      38.598  37.083  10.420  1.00 16.13           C
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ATOM	1463	CZ	PHE	A	94	39.255	36.106	9.711	1.00	15.54	C
ATOM	1465	CE2	PHE	A	94	39.660	34.948	10.345	1.00	16.06	C
ATOM	1467	CD2	PHE	A	94	39.409	34.768	11.682	1.00	13.20	C
ATOM	1469	C	PHE	A	94	36.984	34.837	15.681	1.00	12.26	C
ATOM	1470	O	PHE	A	94	36.882	35.794	16.458	1.00	11.72	O
ATOM	1471	N	VAL	A	95	36.998	33.581	16.097	1.00	12.66	N
ATOM	1473	CA	VAL	A	95	37.063	33.304	17.530	1.00	13.63	C
ATOM	1475	CB	VAL	A	95	36.390	31.976	17.924	1.00	13.74	C
ATOM	1477	CG1	VAL	A	95	37.054	30.809	17.246	1.00	15.18	C
ATOM	1481	CG2	VAL	A	95	36.397	31.814	19.473	1.00	15.16	C
ATOM	1485	C	VAL	A	95	38.554	33.362	17.882	1.00	13.85	C
ATOM	1486	O	VAL	A	95	39.399	32.777	17.203	1.00	13.33	O
ATOM	1487	N	VAL	A	96	38.862	34.116	18.924	1.00	14.98	N
ATOM	1489	CA	VAL	A	96	40.231	34.355	19.364	1.00	16.25	C
ATOM	1491	CB	VAL	A	96	40.478	35.868	19.524	1.00	16.70	C
ATOM	1493	CG1	VAL	A	96	41.904	36.141	20.002	1.00	17.91	C
ATOM	1497	CG2	VAL	A	96	40.202	36.576	18.209	1.00	17.10	C
ATOM	1501	C	VAL	A	96	40.466	33.634	20.691	1.00	17.27	C
ATOM	1502	O	VAL	A	96	39.695	33.788	21.641	1.00	16.34	O
ATOM	1503	N	ILE	A	97	41.522	32.830	20.726	1.00	18.96	N
ATOM	1505	CA	ILE	A	97	41.842	32.001	21.883	1.00	20.35	C
ATOM	1507	CB	ILE	A	97	42.745	30.808	21.453	1.00	20.22	C
ATOM	1509	CG1	ILE	A	97	42.107	30.040	20.289	1.00	19.14	C
ATOM	1512	CD1	ILE	A	97	40.750	29.510	20.588	1.00	18.75	C
ATOM	1516	CG2	ILE	A	97	43.031	29.889	22.639	1.00	20.66	C
ATOM	1520	C	ILE	A	97	42.545	32.863	22.925	1.00	22.26	C
ATOM	1521	O	ILE	A	97	43.457	33.599	22.579	1.00	22.69	O
ATOM	1522	N	PRO	A	98	42.099	32.814	24.176	1.00	24.62	N
ATOM	1523	CA	PRO	A	98	42.733	33.599	25.240	1.00	26.52	C
ATOM	1525	CB	PRO	A	98	41.778	33.441	26.426	1.00	25.97	C
ATOM	1528	CG	PRO	A	98	40.990	32.213	26.153	1.00	25.73	C
ATOM	1531	CD	PRO	A	98	40.942	32.054	24.669	1.00	24.63	C
ATOM	1534	C	PRO	A	98	44.114	33.108	25.621	1.00	28.91	C
ATOM	1535	O	PRO	A	98	44.507	31.975	25.313	1.00	28.13	O
ATOM	1536	N	ASP	A	99	44.838	33.999	26.295	1.00	32.98	N
ATOM	1538	CA	ASP	A	99	46.175	33.735	26.804	1.00	34.16	C
ATOM	1540	CB	ASP	A	99	46.090	32.683	27.897	1.00	34.51	C
ATOM	1543	CG	ASP	A	99	45.238	33.162	29.064	1.00	36.30	C
ATOM	1544	OD1	ASP	A	99	45.547	34.247	29.608	1.00	39.17	O
ATOM	1545	OD2	ASP	A	99	44.222	32.559	29.481	1.00	39.73	O
ATOM	1546	C	ASP	A	99	47.083	33.361	25.645	1.00	35.01	C
ATOM	1547	O	ASP	A	99	47.925	32.468	25.733	1.00	35.21	O
ATOM	1548	N	THR	A	100	46.914	34.118	24.568	1.00	36.50	N
ATOM	1550	CA	THR	A	100	47.559	33.836	23.307	1.00	35.94	C
ATOM	1552	CB	THR	A	100	46.536	33.051	22.457	1.00	36.18	C
ATOM	1554	OG1	THR	A	100	47.151	31.891	21.882	1.00	36.31	O
ATOM	1556	CG2	THR	A	100	46.021	33.845	21.282	1.00	35.16	C
ATOM	1560	C	THR	A	100	48.034	35.115	22.606	1.00	36.08	C
ATOM	1561	O	THR	A	100	48.650	35.058	21.542	1.00	35.70	O
ATOM	1562	N	GLY	A	101	47.776	36.267	23.220	1.00	36.43	N
ATOM	1564	CA	GLY	A	101	48.151	37.550	22.636	1.00	36.52	C
ATOM	1567	C	GLY	A	101	47.365	37.850	21.368	1.00	36.69	C
ATOM	1568	O	GLY	A	101	47.842	38.551	20.467	1.00	36.66	O
ATOM	1569	N	ASN	A	102	46.149	37.309	21.305	1.00	37.04	N
ATOM	1571	CA	ASN	A	102	45.279	37.436	20.135	1.00	35.46	C
ATOM	1573	CB	ASN	A	102	44.881	38.904	19.889	1.00	35.74	C
ATOM	1576	CG	ASN	A	102	44.070	39.509	21.044	1.00	36.19	C
ATOM	1577	OD1	ASN	A	102	43.556	38.795	21.914	1.00	36.82	O
ATOM	1578	ND2	ASN	A	102	43.948	40.836	21.046	1.00	38.45	N
ATOM	1581	C	ASN	A	102	45.884	36.813	18.855	1.00	34.28	C
ATOM	1582	O	ASN	A	102	45.401	37.081	17.757	1.00	34.09	O
ATOM	1583	N	SER	A	103	46.919	35.978	18.997	1.00	32.94	N
ATOM	1585	CA	SER	A	103	47.608	35.359	17.852	1.00	30.99	C

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ATOM   1587  CB  SER A 103      49.068  35.070  18.214  1.00 31.29           C
ATOM   1590  OG  SER A 103      49.175  34.552  19.532  1.00 32.26           O
ATOM   1592  C   SER A 103      46.981  34.072  17.315  1.00 28.93           C
ATOM   1593  O   SER A 103      47.135  33.752  16.140  1.00 29.01           O
ATOM   1594  N   GLU A 104      46.308  33.320  18.173  1.00 26.74           N
ATOM   1596  CA  GLU A 104      45.648  32.098  17.739  1.00 23.55           C
ATOM   1598  CB  GLU A 104      45.821  30.969  18.759  1.00 23.10           C
ATOM   1601  CG  GLU A 104      45.217  29.652  18.294  1.00 22.29           C
ATOM   1604  CD  GLU A 104      45.267  28.539  19.335  1.00 20.79           C
ATOM   1605  OE1 GLU A 104      44.705  27.459  19.063  1.00 18.27           O
ATOM   1606  OE2 GLU A 104      45.872  28.735  20.405  1.00 19.73           O
ATOM   1607  C   GLU A 104      44.166  32.431  17.527  1.00 21.26           C
ATOM   1608  O   GLU A 104      43.463  32.788  18.468  1.00 20.20           O
ATOM   1609  N   GLU A 105      43.706  32.342  16.286  1.00 19.27           N
ATOM   1611  CA  GLU A 105      42.310  32.652  15.989  1.00 17.92           C
ATOM   1613  CB  GLU A 105      42.119  34.141  15.658  1.00 18.34           C
ATOM   1616  CG  GLU A 105      42.614  34.515  14.283  1.00 19.41           C
ATOM   1619  CD  GLU A 105      42.443  35.986  13.960  1.00 21.46           C
ATOM   1620  OE1 GLU A 105      42.657  36.346  12.779  1.00 22.55           O
ATOM   1621  OE2 GLU A 105      42.097  36.770  14.872  1.00 19.79           O
ATOM   1622  C   GLU A 105      41.807  31.788  14.851  1.00 16.31           C
ATOM   1623  O   GLU A 105      42.589  31.268  14.050  1.00 16.35           O
ATOM   1624  N   TYR A 106      40.489  31.642  14.779  1.00 14.92           N
ATOM   1626  CA  TYR A 106      39.856  30.789  13.784  1.00 13.70           C
ATOM   1628  CB  TYR A 106      39.466  29.416  14.400  1.00 12.91           C
ATOM   1631  CG  TYR A 106      40.630  28.705  15.037  1.00 13.45           C
ATOM   1632  CD1 TYR A 106      41.441  27.863  14.296  1.00 15.31           C
ATOM   1634  CE1 TYR A 106      42.517  27.226  14.869  1.00 15.32           C
ATOM   1636  CZ  TYR A 106      42.812  27.428  16.186  1.00 15.25           C
ATOM   1637  OH  TYR A 106      43.904  26.776  16.728  1.00 16.78           O
ATOM   1639  CE2 TYR A 106      42.027  28.251  16.961  1.00 15.05           C
ATOM   1641  CD2 TYR A 106      40.934  28.890  16.379  1.00 13.75           C
ATOM   1643  C   TYR A 106      38.605  31.460  13.230  1.00 13.16           C
ATOM   1644  O   TYR A 106      37.789  31.993  14.001  1.00 12.90           O
ATOM   1645  N   ILE A 107      38.432  31.416  11.911  1.00 12.87           N
ATOM   1647  CA  ILE A 107      37.219  31.954  11.296  1.00 13.04           C
ATOM   1649  CB  ILE A 107      37.271  31.865   9.734  1.00 13.61           C
ATOM   1651  CG1 ILE A 107      36.049  32.531   9.105  1.00 16.08           C
ATOM   1654  CD1 ILE A 107      36.054  33.996   9.131  1.00 19.25           C
ATOM   1658  CG2 ILE A 107      37.277  30.431   9.234  1.00 14.39           C
ATOM   1662  C   ILE A 107      36.026  31.203  11.890  1.00 12.69           C
ATOM   1663  O   ILE A 107      36.050  29.967  11.991  1.00 12.26           O
ATOM   1664  N   ILE A 108      34.994  31.931  12.314  1.00 10.96           N
ATOM   1666  CA  ILE A 108      33.831  31.283  12.892  1.00 11.58           C
ATOM   1668  CB  ILE A 108      33.823  31.470  14.438  1.00 11.47           C
ATOM   1670  CG1 ILE A 108      32.825  30.527  15.117  1.00 12.45           C
ATOM   1673  CD1 ILE A 108      33.138  29.042  14.913  1.00 14.07           C
ATOM   1677  CG2 ILE A 108      33.541  32.903  14.825  1.00 11.20           C
ATOM   1681  C   ILE A 108      32.516  31.695  12.234  1.00 11.67           C
ATOM   1682  O   ILE A 108      31.510  31.041  12.437  1.00 13.19           O
ATOM   1683  N   ALA A 109      32.512  32.756  11.438  1.00 11.35           N
ATOM   1685  CA  ALA A 109      31.319  33.139  10.675  1.00 12.23           C
ATOM   1687  CB  ALA A 109      30.290  33.798  11.582  1.00 12.13           C
ATOM   1691  C   ALA A 109      31.699  34.113   9.557  1.00 12.08           C
ATOM   1692  O   ALA A 109      32.648  34.879   9.714  1.00 12.05           O
ATOM   1693  N   GLU A 110      30.956  34.090   8.448  1.00 13.59           N
ATOM   1695  CA  GLU A 110      31.147  35.085   7.399  1.00 13.30           C
ATOM   1697  CB  GLU A 110      32.149  34.594   6.336  1.00 14.11           C
ATOM   1700  CG  GLU A 110      32.258  35.509   5.123  1.00 15.16           C
ATOM   1703  CD  GLU A 110      33.187  34.947   4.059  1.00 18.76           C
ATOM   1704  OE1 GLU A 110      34.393  35.270   4.085  1.00 20.47           O
ATOM   1705  OE2 GLU A 110      32.706  34.165   3.204  1.00 21.90           O
ATOM   1706  C   GLU A 110      29.814  35.470   6.762  1.00 13.12           C
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ATOM   1707  O   GLU A 110      29.028  34.608   6.372  1.00 13.60           O
ATOM   1708  N   TRP A 111      29.559  36.772   6.709  1.00 12.93           N
ATOM   1710  CA  TRP A 111      28.420  37.335   6.032  1.00 14.60           C
ATOM   1712  CB  TRP A 111      27.809  38.507   6.808  1.00 15.14           C
ATOM   1715  CG  TRP A 111      26.726  39.127   5.988  1.00 15.69           C
ATOM   1716  CD1 TRP A 111      26.858  40.164   5.110  1.00 16.10           C
ATOM   1718  NE1 TRP A 111      25.661  40.419   4.489  1.00 16.03           N
ATOM   1720  CE2 TRP A 111      24.727  39.530   4.949  1.00 18.16           C
ATOM   1721  CD2 TRP A 111      25.367  38.695   5.891  1.00 18.12           C
ATOM   1722  CE3 TRP A 111      24.618  37.689   6.510  1.00 19.20           C
ATOM   1724  CZ3 TRP A 111      23.277  37.560   6.189  1.00 21.05           C
ATOM   1726  CH2 TRP A 111      22.673  38.410   5.255  1.00 20.77           C
ATOM   1728  CZ2 TRP A 111      23.382  39.397   4.626  1.00 19.73           C
ATOM   1730  C   TRP A 111      28.912  37.854   4.684  1.00 16.60           C
ATOM   1731  O   TRP A 111      29.813  38.692   4.621  1.00 15.60           O
ATOM   1732  N   LYS A 112      28.344  37.323   3.618  1.00 18.75           N
ATOM   1734  CA  LYS A 112      28.630  37.843   2.288  1.00 21.69           C
ATOM   1736  CB  LYS A 112      29.829  37.176   1.651  1.00 21.83           C
ATOM   1739  CG  LYS A 112      30.317  37.972   0.444  1.00 24.07           C
ATOM   1742  CD  LYS A 112      31.330  37.217  -0.360  1.00 26.81           C
ATOM   1745  CE  LYS A 112      32.648  37.120   0.351  1.00 28.67           C
ATOM   1748  NZ  LYS A 112      33.684  36.627  -0.601  1.00 31.60           N
ATOM   1752  C   LYS A 112      27.394  37.622   1.452  1.00 23.68           C
ATOM   1753  O   LYS A 112      27.097  36.495   1.042  1.00 24.51           O
ATOM   1754  N   LYS A 113      26.678  38.708   1.226  1.00 26.48           N
ATOM   1756  CA  LYS A 113      25.423  38.686   0.536  1.00 29.15           C
ATOM   1758  CB  LYS A 113      24.840  40.091   0.501  1.00 29.56           C
ATOM   1761  CG  LYS A 113      23.349  40.115   0.396  1.00 31.24           C
ATOM   1764  CD  LYS A 113      22.790  41.488   0.542  1.00 33.22           C
ATOM   1767  CE  LYS A 113      21.264  41.332   0.752  1.00 34.56           C
ATOM   1770  NZ  LYS A 113      20.811  40.425   1.911  1.00 34.61           N
ATOM   1774  C   LYS A 113      25.589  38.215  -0.870  1.00 30.92           C
ATOM   1775  O   LYS A 113      26.581  38.511  -1.536  1.00 31.06           O
ATOM   1776  N   ALA A 114      24.575  37.490  -1.308  1.00 33.51           N
ATOM   1778  CA  ALA A 114      24.484  37.015  -2.669  1.00 34.75           C
ATOM   1780  CB  ALA A 114      25.336  35.792  -2.859  1.00 35.33           C
ATOM   1784  C   ALA A 114      23.016  36.693  -2.918  1.00 35.91           C
ATOM   1785  O   ALA A 114      22.275  36.373  -1.973  1.00 37.33           O
ATOM   1786  O   ACE B   0      45.942  19.784  14.579  1.00 39.31           O
ATOM   1787  C   ACE B   0      45.727  19.383  15.830  1.00 38.58           C
ATOM   1788  CA  ACE B   0      44.966  18.078  16.167  1.00 38.68           C
ATOM   1789  N   SER B   1      45.689  20.569  16.659  1.00 19.77           N
ATOM   1791  CA  SER B   1      45.431  20.583  18.122  1.00 17.98           C
ATOM   1793  CB  SER B   1      45.842  21.915  18.761  1.00 18.61           C
ATOM   1796  OG  SER B   1      44.965  22.977  18.387  1.00 16.99           O
ATOM   1798  C   SER B   1      43.950  20.368  18.350  1.00 17.78           C
ATOM   1799  O   SER B   1      43.169  20.531  17.414  1.00 17.20           O
ATOM   1802  N   ALA B   2      43.575  19.905  19.539  1.00 16.87           N
ATOM   1804  CA  ALA B   2      42.161  19.821  19.902  1.00 16.13           C
ATOM   1806  CB  ALA B   2      41.991  19.439  21.370  1.00 16.68           C
ATOM   1810  C   ALA B   2      41.405  21.112  19.611  1.00 15.41           C
ATOM   1811  O   ALA B   2      40.278  21.088  19.118  1.00 14.56           O
ATOM   1812  N   THR B   3      42.018  22.234  19.952  1.00 15.22           N
ATOM   1814  CA  THR B   3      41.391  23.526  19.766  1.00 14.31           C
ATOM   1816  CB  THR B   3      42.264  24.598  20.403  1.00 14.74           C
ATOM   1818  OG1 THR B   3      42.272  24.402  21.826  1.00 16.14           O
ATOM   1820  CG2 THR B   3      41.660  25.961  20.217  1.00 14.49           C
ATOM   1824  C   THR B   3      41.194  23.813  18.295  1.00 13.68           C
ATOM   1825  O   THR B   3      40.111  24.227  17.861  1.00 13.01           O
ATOM   1826  N   SER B   4      42.231  23.568  17.505  1.00 12.92           N
ATOM   1828  CA  SER B   4      42.114  23.823  16.074  1.00 12.63           C
ATOM   1830  CB  SER B   4      43.466  23.637  15.389  1.00 13.31           C
ATOM   1833  OG  SER B   4      43.349  23.779  13.980  1.00 15.34           O
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ATOM   1835  C   SER B   4      41.042  22.939  15.427  1.00 11.99           C
ATOM   1836  O   SER B   4      40.232  23.408  14.613  1.00 11.15           O
ATOM   1837  N   LEU B   5      41.045  21.652  15.755  1.00 11.62           N
ATOM   1839  CA  LEU B   5      40.036  20.744  15.224  1.00 10.95           C
ATOM   1841  CB  LEU B   5      40.253  19.326  15.755  1.00 11.58           C
ATOM   1844  CG  LEU B   5      41.493  18.602  15.191  1.00 13.26           C
ATOM   1846  CD1 LEU B   5      41.671  17.274  15.878  1.00 14.30           C
ATOM   1850  CD2 LEU B   5      41.430  18.388  13.679  1.00 16.20           C
ATOM   1854  C   LEU B   5      38.633  21.207  15.621  1.00 10.05           C
ATOM   1855  O   LEU B   5      37.713  21.115  14.830  1.00  9.60           O
ATOM   1856  N   THR B   6      38.482  21.669  16.858  1.00  9.83           N
ATOM   1858  CA  THR B   6      37.187  22.108  17.359  1.00 10.01           C
ATOM   1860  CB  THR B   6      37.300  22.622  18.794  1.00  9.92           C
ATOM   1862  OG1 THR B   6      37.622  21.536  19.681  1.00 10.43           O
ATOM   1864  CG2 THR B   6      35.965  23.168  19.289  1.00  9.67           C
ATOM   1868  C   THR B   6      36.616  23.197  16.490  1.00 10.19           C
ATOM   1869  O   THR B   6      35.478  23.121  16.047  1.00 10.13           O
ATOM   1870  N   PHE B   7      37.416  24.217  16.232  1.00 10.95           N
ATOM   1872  CA  PHE B   7      36.898  25.372  15.532  1.00 10.37           C
ATOM   1874  CB  PHE B   7      37.576  26.643  16.024  1.00 10.33           C
ATOM   1877  CG  PHE B   7      37.149  27.021  17.415  1.00 10.12           C
ATOM   1878  CD1 PHE B   7      35.833  27.366  17.673  1.00 10.88           C
ATOM   1880  CE1 PHE B   7      35.417  27.659  18.945  1.00 11.19           C
ATOM   1882  CZ  PHE B   7      36.296  27.605  19.969  1.00 11.18           C
ATOM   1884  CE2 PHE B   7      37.605  27.245  19.734  1.00 12.59           C
ATOM   1886  CD2 PHE B   7      38.021  26.936  18.466  1.00 11.83           C
ATOM   1888  C   PHE B   7      36.909  25.194  14.025  1.00 10.94           C
ATOM   1889  O   PHE B   7      36.103  25.820  13.353  1.00 11.93           O
ATOM   1890  N   GLN B   8      37.767  24.329  13.489  1.00 11.93           N
ATOM   1892  CA  GLN B   8      37.647  24.010  12.067  1.00 11.34           C
ATOM   1894  CB  GLN B   8      38.761  23.087  11.621  1.00 12.48           C
ATOM   1897  CG  GLN B   8      40.113  23.720  11.528  1.00 14.22           C
ATOM   1900  CD  GLN B   8      41.117  22.698  11.051  1.00 15.81           C
ATOM   1901  OE1 GLN B   8      42.036  22.331  11.781  1.00 19.91           O
ATOM   1902  NE2 GLN B   8      40.902  22.184   9.843  1.00 17.92           N
ATOM   1905  C   GLN B   8      36.316  23.286  11.855  1.00 10.37           C
ATOM   1906  O   GLN B   8      35.580  23.546  10.908  1.00 10.98           O
ATOM   1907  N   LEU B   9      36.006  22.360  12.758  1.00 10.06           N
ATOM   1909  CA  LEU B   9      34.757  21.608  12.648  1.00  9.71           C
ATOM   1911  CB  LEU B   9      34.726  20.455  13.634  1.00  9.51           C
ATOM   1914  CG  LEU B   9      33.493  19.574  13.606  1.00 10.41           C
ATOM   1916  CD1 LEU B   9      33.447  18.825  12.265  1.00 11.23           C
ATOM   1920  CD2 LEU B   9      33.561  18.587  14.753  1.00  9.36           C
ATOM   1924  C   LEU B   9      33.552  22.498  12.880  1.00  9.33           C
ATOM   1925  O   LEU B   9      32.566  22.409  12.160  1.00 10.21           O
ATOM   1926  N   ALA B  10      33.618  23.376  13.874  1.00  9.78           N
ATOM   1928  CA  ALA B  10      32.476  24.246  14.138  1.00  9.75           C
ATOM   1930  CB  ALA B  10      32.727  25.091  15.353  1.00 10.03           C
ATOM   1934  C   ALA B  10      32.145  25.126  12.919  1.00  9.57           C
ATOM   1935  O   ALA B  10      30.982  25.275  12.554  1.00  8.63           O
ATOM   1936  N   TYR B  11      33.155  25.688  12.269  1.00  9.75           N
ATOM   1938  CA  TYR B  11      32.885  26.566  11.136  1.00 10.72           C
ATOM   1940  CB  TYR B  11      34.159  27.237  10.688  1.00 10.48           C
ATOM   1943  CG  TYR B  11      33.979  28.188   9.535  1.00 11.27           C
ATOM   1944  CD1 TYR B  11      34.664  27.988   8.352  1.00 11.30           C
ATOM   1946  CE1 TYR B  11      34.534  28.867   7.292  1.00 13.85           C
ATOM   1948  CZ  TYR B  11      33.706  29.949   7.409  1.00 14.97           C
ATOM   1949  OH  TYR B  11      33.579  30.827   6.350  1.00 16.32           O
ATOM   1951  CE2 TYR B  11      33.022  30.183   8.582  1.00 14.29           C
ATOM   1953  CD2 TYR B  11      33.159  29.300   9.640  1.00 12.05           C
ATOM   1955  C   TYR B  11      32.250  25.801   9.987  1.00 11.09           C
ATOM   1956  O   TYR B  11      31.380  26.310   9.272  1.00 11.19           O
ATOM   1957  N   LEU B  12      32.672  24.556   9.823  1.00 12.21           N
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	ATOM	1959	CA	LEU	B	12	32.179	23.730	8.742	1.00	12.45	C
	ATOM	1961	CB	LEU	B	12	33.187	22.614	8.470	1.00	12.95	C
	ATOM	1964	CG	LEU	B	12	33.011	21.779	7.209	1.00	16.64	C
	ATOM	1966	CD1	LEU	B	12	32.907	22.662	5.962	1.00	17.27	C
5	ATOM	1970	CD2	LEU	B	12	34.193	20.804	7.093	1.00	18.06	C
	ATOM	1974	C	LEU	B	12	30.767	23.156	8.956	1.00	12.37	C
	ATOM	1975	O	LEU	B	12	29.914	23.302	8.071	1.00	13.09	O
	ATOM	1976	N	VAL	B	13	30.514	22.525	10.103	1.00	12.47	N
	ATOM	1978	CA	VAL	B	13	29.256	21.805	10.335	1.00	12.51	C
10	ATOM	1980	CB	VAL	B	13	29.485	20.381	10.894	1.00	13.37	C
	ATOM	1982	CG1	VAL	B	13	30.492	19.620	10.042	1.00	15.30	C
	ATOM	1986	CG2	VAL	B	13	29.922	20.398	12.336	1.00	14.08	C
	ATOM	1990	C	VAL	B	13	28.273	22.541	11.237	1.00	12.00	C
	ATOM	1991	O	VAL	B	13	27.106	22.172	11.319	1.00	11.68	O
15	ATOM	1992	N	LYS	B	14	28.775	23.558	11.917	1.00	11.40	N
	ATOM	1994	CA	LYS	B	14	27.991	24.459	12.764	1.00	11.19	C
	ATOM	1996	CB	LYS	B	14	26.759	25.042	12.035	1.00	11.48	C
	ATOM	1999	CG	LYS	B	14	27.039	25.761	10.731	1.00	11.14	C
	ATOM	2002	CD	LYS	B	14	28.190	26.750	10.824	1.00	9.92	C
20	ATOM	2005	CE	LYS	B	14	28.422	27.540	9.526	1.00	10.31	C
	ATOM	2008	NZ	LYS	B	14	29.602	28.448	9.653	1.00	12.24	N
	ATOM	2012	C	LYS	B	14	27.549	23.850	14.104	1.00	10.98	C
	ATOM	2013	O	LYS	B	14	27.795	24.432	15.165	1.00	10.55	O
	ATOM	2014	N	LYS	B	15	26.914	22.684	14.069	1.00	10.86	N
25	ATOM	2016	CA	LYS	B	15	26.282	22.127	15.256	1.00	12.66	C
	ATOM	2018	CB	LYS	B	15	24.753	22.337	15.149	1.00	13.78	C
	ATOM	2021	CG	LYS	B	15	23.878	21.739	16.237	1.00	18.00	C
	ATOM	2024	CD	LYS	B	15	22.382	22.146	16.068	1.00	23.19	C
	ATOM	2027	CE	LYS	B	15	21.671	21.394	14.921	1.00	27.17	C
30	ATOM	2030	NZ	LYS	B	15	20.279	21.902	14.547	1.00	34.39	N
	ATOM	2034	C	LYS	B	15	26.637	20.640	15.355	1.00	12.47	C
	ATOM	2035	O	LYS	B	15	26.526	19.913	14.363	1.00	13.03	O
	ATOM	2036	N	ILE	B	16	27.094	20.207	16.526	1.00	13.16	N
	ATOM	2038	CA	ILE	B	16	27.368	18.784	16.760	1.00	12.11	C
35	ATOM	2040	CB	ILE	B	16	28.707	18.380	16.149	1.00	12.61	C
	ATOM	2042	CG1	ILE	B	16	28.660	16.894	15.756	1.00	13.09	C
	ATOM	2045	CD1	ILE	B	16	29.822	16.447	14.941	1.00	14.91	C
	ATOM	2049	CG2	ILE	B	16	29.831	18.704	17.105	1.00	12.04	C
	ATOM	2053	C	ILE	B	16	27.276	18.487	18.258	1.00	12.09	C
40	ATOM	2054	O	ILE	B	16	27.516	19.360	19.098	1.00	11.30	O
	ATOM	2055	N	ASP	B	17	26.903	17.257	18.587	1.00	11.37	N
	ATOM	2057	CA	ASP	B	17	26.701	16.850	19.977	1.00	11.97	C
	ATOM	2059	CB	ASP	B	17	25.238	17.060	20.347	1.00	12.19	C
	ATOM	2062	CG	ASP	B	17	24.929	16.770	21.795	1.00	15.47	C
45	ATOM	2063	OD1	ASP	B	17	25.834	16.488	22.602	1.00	15.71	O
	ATOM	2064	OD2	ASP	B	17	23.746	16.838	22.212	1.00	21.94	O
	ATOM	2065	C	ASP	B	17	27.026	15.373	20.040	1.00	11.79	C
	ATOM	2066	O	ASP	B	17	26.246	14.552	19.554	1.00	12.85	O
	ATOM	2067	N	PHE	B	18	28.190	15.029	20.566	1.00	10.57	N
50	ATOM	2069	CA	PHE	B	18	28.552	13.620	20.686	1.00	10.47	C
	ATOM	2071	CB	PHE	B	18	29.385	13.115	19.479	1.00	9.71	C
	ATOM	2074	CG	PHE	B	18	30.728	13.797	19.316	1.00	9.88	C
	ATOM	2075	CD1	PHE	B	18	31.732	13.663	20.275	1.00	7.77	C
	ATOM	2077	CE1	PHE	B	18	32.936	14.318	20.131	1.00	9.85	C
55	ATOM	2079	CZ	PHE	B	18	33.172	15.094	19.013	1.00	10.47	C
	ATOM	2081	CE2	PHE	B	18	32.194	15.226	18.062	1.00	9.60	C
	ATOM	2083	CD2	PHE	B	18	30.979	14.584	18.211	1.00	9.21	C
	ATOM	2085	C	PHE	B	18	29.281	13.324	21.983	1.00	9.72	C
	ATOM	2086	O	PHE	B	18	29.760	14.220	22.691	1.00	10.09	O
60	ATOM	2087	N	ASP	B	19	29.326	12.041	22.306	1.00	9.08	N
	ATOM	2089	CA	ASP	B	19	30.050	11.562	23.459	1.00	9.72	C
	ATOM	2091	CB	ASP	B	19	29.193	11.549	24.716	1.00	9.54	C
	ATOM	2094	CG	ASP	B	19	29.999	11.225	25.937	1.00	11.36	C

	ATOM	2095	OD1	ASP	B	19	29.498	11.408	27.093	1.00	13.80	O
	ATOM	2096	OD2	ASP	B	19	31.149	10.767	25.834	1.00	10.64	O
	ATOM	2097	C	ASP	B	19	30.543	10.155	23.160	1.00	9.57	C
	ATOM	2098	O	ASP	B	19	29.765	9.177	23.218	1.00	10.53	O
5	ATOM	2099	N	TYR	B	20	31.830	10.085	22.813	1.00	9.01	N
	ATOM	2101	CA	TYR	B	20	32.505	8.831	22.518	1.00	8.11	C
	ATOM	2103	CB	TYR	B	20	33.232	8.896	21.163	1.00	7.80	C
	ATOM	2106	CG	TYR	B	20	32.292	8.800	19.966	1.00	7.18	C
	ATOM	2107	CD1	TYR	B	20	31.765	9.933	19.381	1.00	8.23	C
10	ATOM	2109	CE1	TYR	B	20	30.897	9.849	18.286	1.00	8.63	C
	ATOM	2111	CZ	TYR	B	20	30.570	8.623	17.777	1.00	8.76	C
	ATOM	2112	OH	TYR	B	20	29.690	8.504	16.698	1.00	7.11	O
	ATOM	2114	CE2	TYR	B	20	31.081	7.492	18.358	1.00	7.59	C
	ATOM	2116	CD2	TYR	B	20	31.929	7.580	19.435	1.00	8.21	C
15	ATOM	2118	C	TYR	B	20	33.453	8.446	23.657	1.00	7.82	C
	ATOM	2119	O	TYR	B	20	34.453	7.777	23.416	1.00	8.72	O
	ATOM	2120	N	THR	B	21	33.128	8.834	24.892	1.00	8.34	N
	ATOM	2122	CA	THR	B	21	33.897	8.365	26.046	1.00	8.81	C
	ATOM	2124	CB	THR	B	21	33.320	8.922	27.334	1.00	9.96	C
20	ATOM	2126	OG1	THR	B	21	33.372	10.363	27.299	1.00	10.43	O
	ATOM	2128	CG2	THR	B	21	34.222	8.512	28.491	1.00	9.86	C
	ATOM	2132	C	THR	B	21	33.804	6.831	26.040	1.00	9.35	C
	ATOM	2133	O	THR	B	21	32.692	6.291	26.011	1.00	9.22	O
	ATOM	2134	N	PRO	B	22	34.922	6.111	26.025	1.00	9.19	N
25	ATOM	2135	CA	PRO	B	22	34.844	4.647	25.905	1.00	9.50	C
	ATOM	2137	CB	PRO	B	22	36.209	4.280	25.352	1.00	9.82	C
	ATOM	2140	CG	PRO	B	22	37.138	5.334	25.941	1.00	9.26	C
	ATOM	2143	CD	PRO	B	22	36.320	6.589	26.015	1.00	9.34	C
	ATOM	2146	C	PRO	B	22	34.616	3.930	27.227	1.00	10.16	C
30	ATOM	2147	O	PRO	B	22	35.520	3.898	28.070	1.00	10.91	O
	ATOM	2148	N	ASN	B	23	33.413	3.394	27.413	1.00	10.40	N
	ATOM	2150	CA	ASN	B	23	33.082	2.645	28.614	1.00	10.53	C
	ATOM	2152	CB	ASN	B	23	31.680	3.014	29.089	1.00	11.14	C
	ATOM	2155	CG	ASN	B	23	31.595	4.472	29.590	1.00	13.44	C
35	ATOM	2156	OD1	ASN	B	23	31.816	4.721	30.763	1.00	19.39	O
	ATOM	2157	ND2	ASN	B	23	31.342	5.441	28.685	1.00	14.28	N
	ATOM	2160	C	ASN	B	23	33.228	1.143	28.312	1.00	9.85	C
	ATOM	2161	O	ASN	B	23	32.489	0.595	27.483	1.00	10.11	O
	ATOM	2162	N	TRP	B	24	34.208	0.502	28.942	1.00	8.96	N
40	ATOM	2164	CA	TRP	B	24	34.524	-0.899	28.684	1.00	8.99	C
	ATOM	2166	CB	TRP	B	24	36.032	-1.117	28.760	1.00	8.98	C
	ATOM	2169	CG	TRP	B	24	36.799	-0.256	27.823	1.00	8.22	C
	ATOM	2170	CD1	TRP	B	24	37.375	0.965	28.116	1.00	9.52	C
	ATOM	2172	NE1	TRP	B	24	38.003	1.469	27.000	1.00	9.37	N
45	ATOM	2174	CE2	TRP	B	24	37.860	0.574	25.967	1.00	9.60	C
	ATOM	2175	CD2	TRP	B	24	37.099	-0.517	26.446	1.00	9.21	C
	ATOM	2176	CE3	TRP	B	24	36.824	-1.577	25.560	1.00	9.09	C
	ATOM	2178	CZ3	TRP	B	24	37.292	-1.502	24.264	1.00	9.03	C
	ATOM	2180	CH2	TRP	B	24	38.045	-0.414	23.829	1.00	7.76	C
50	ATOM	2182	CZ2	TRP	B	24	38.337	0.636	24.661	1.00	8.73	C
	ATOM	2184	C	TRP	B	24	33.806	-1.771	29.715	1.00	10.09	C
	ATOM	2185	O	TRP	B	24	34.024	-1.610	30.922	1.00	11.35	O
	ATOM	2186	N	GLY	B	25	32.931	-2.655	29.234	1.00	10.43	N
	ATOM	2188	CA	GLY	B	25	32.143	-3.516	30.098	1.00	9.95	C
55	ATOM	2191	C	GLY	B	25	32.749	-4.894	30.176	1.00	10.10	C
	ATOM	2192	O	GLY	B	25	33.220	-5.444	29.184	1.00	10.44	O
	ATOM	2193	N	ARG	B	26	32.721	-5.470	31.372	1.00	9.91	N
	ATOM	2195	CA	ARG	B	26	33.393	-6.738	31.594	1.00	10.74	C
	ATOM	2197	CB	ARG	B	26	34.185	-6.692	32.897	1.00	11.28	C
60	ATOM	2200	CG	ARG	B	26	35.418	-5.792	32.764	1.00	14.61	C
	ATOM	2203	CD	ARG	B	26	36.168	-5.505	34.025	1.00	18.47	C
	ATOM	2206	NE	ARG	B	26	37.353	-4.694	33.713	1.00	22.81	N
	ATOM	2208	CZ	ARG	B	26	38.526	-5.167	33.274	1.00	20.92	C

	ATOM	2209	NH1	ARG	B	26	38.730	-6.471	33.093	1.00	16.47	N
	ATOM	2212	NH2	ARG	B	26	39.522	-4.318	33.028	1.00	21.83	N
	ATOM	2215	C	ARG	B	26	32.431	-7.898	31.604	1.00	11.12	C
	ATOM	2216	O	ARG	B	26	31.227	-7.721	31.802	1.00	11.55	O
5	ATOM	2217	N	GLY	B	27	32.990	-9.082	31.405	1.00	10.76	N
	ATOM	2219	CA	GLY	B	27	32.203	-10.305	31.337	1.00	11.53	C
	ATOM	2222	C	GLY	B	27	32.382	-11.170	32.553	1.00	12.49	C
	ATOM	2223	O	GLY	B	27	32.588	-10.677	33.664	1.00	11.38	O
	ATOM	2224	N	THR	B	28	32.285	-12.468	32.308	1.00	14.02	N
10	ATOM	2226	CA	THR	B	28	32.361	-13.494	33.325	1.00	14.82	C
	ATOM	2228	CB	THR	B	28	30.965	-14.118	33.495	1.00	14.80	C
	ATOM	2230	OG1	THR	B	28	30.052	-13.151	34.032	1.00	15.92	O
	ATOM	2232	CG2	THR	B	28	30.974	-15.235	34.519	1.00	16.05	C
	ATOM	2236	C	THR	B	28	33.327	-14.552	32.828	1.00	15.01	C
15	ATOM	2237	O	THR	B	28	33.037	-15.215	31.838	1.00	15.21	O
	ATOM	2238	N	PRO	B	29	34.490	-14.700	33.454	1.00	16.30	N
	ATOM	2239	CA	PRO	B	29	34.948	-13.877	34.569	1.00	16.04	C
	ATOM	2241	CB	PRO	B	29	36.219	-14.575	35.028	1.00	16.47	C
	ATOM	2244	CG	PRO	B	29	36.636	-15.376	33.917	1.00	16.57	C
20	ATOM	2247	CD	PRO	B	29	35.467	-15.724	33.083	1.00	16.58	C
	ATOM	2250	C	PRO	B	29	35.286	-12.472	34.156	1.00	15.67	C
	ATOM	2251	O	PRO	B	29	35.396	-12.161	32.977	1.00	14.52	O
	ATOM	2252	N	SER	B	30	35.477	-11.643	35.164	1.00	15.56	N
	ATOM	2254	CA	SER	B	30	35.592	-10.188	34.990	1.00	15.79	C
25	ATOM	2256	CB	SER	B	30	35.368	-9.479	36.338	1.00	16.72	C
	ATOM	2259	OG	SER	B	30	36.454	-9.647	37.224	1.00	19.27	O
	ATOM	2261	C	SER	B	30	36.886	-9.718	34.319	1.00	15.13	C
	ATOM	2262	O	SER	B	30	37.028	-8.535	33.981	1.00	14.89	O
	ATOM	2263	N	SER	B	31	37.813	-10.644	34.117	1.00	15.18	N
30	ATOM	2265	CA	SER	B	31	39.073	-10.365	33.451	1.00	14.92	C
	ATOM	2267	CB	SER	B	31	40.078	-11.489	33.743	1.00	15.53	C
	ATOM	2270	OG	SER	B	31	39.588	-12.751	33.330	1.00	16.71	O
	ATOM	2272	C	SER	B	31	38.833	-10.220	31.958	1.00	14.13	C
	ATOM	2273	O	SER	B	31	39.704	-9.755	31.247	1.00	14.11	O
35	ATOM	2274	N	TYR	B	32	37.655	-10.629	31.492	1.00	13.25	N
	ATOM	2276	CA	TYR	B	32	37.263	-10.430	30.088	1.00	12.84	C
	ATOM	2278	CB	TYR	B	32	36.386	-11.579	29.595	1.00	13.42	C
	ATOM	2281	CG	TYR	B	32	37.194	-12.849	29.494	1.00	17.98	C
	ATOM	2282	CD1	TYR	B	32	37.301	-13.710	30.586	1.00	22.94	C
40	ATOM	2284	CE1	TYR	B	32	38.065	-14.852	30.521	1.00	23.98	C
	ATOM	2286	CZ	TYR	B	32	38.746	-15.143	29.354	1.00	25.27	C
	ATOM	2287	OH	TYR	B	32	39.505	-16.288	29.289	1.00	27.54	O
	ATOM	2289	CE2	TYR	B	32	38.663	-14.298	28.266	1.00	23.63	C
	ATOM	2291	CD2	TYR	B	32	37.895	-13.159	28.344	1.00	21.29	C
45	ATOM	2293	C	TYR	B	32	36.533	-9.112	29.846	1.00	11.60	C
	ATOM	2294	O	TYR	B	32	35.685	-8.695	30.647	1.00	10.60	O
	ATOM	2295	N	ILE	B	33	36.880	-8.444	28.745	1.00	10.45	N
	ATOM	2297	CA	ILE	B	33	36.165	-7.242	28.298	1.00	10.39	C
	ATOM	2299	CB	ILE	B	33	37.144	-6.198	27.724	1.00	10.57	C
50	ATOM	2301	CG1	ILE	B	33	38.031	-5.676	28.860	1.00	13.06	C
	ATOM	2304	CD1	ILE	B	33	39.008	-4.662	28.440	1.00	16.01	C
	ATOM	2308	CG2	ILE	B	33	36.395	-5.062	26.975	1.00	10.92	C
	ATOM	2312	C	ILE	B	33	35.178	-7.715	27.237	1.00	9.65	C
	ATOM	2313	O	ILE	B	33	35.595	-8.249	26.202	1.00	8.88	O
55	ATOM	2314	N	ASP	B	34	33.883	-7.580	27.498	1.00	9.91	N
	ATOM	2316	CA	ASP	B	34	32.857	-8.063	26.583	1.00	9.11	C
	ATOM	2318	CB	ASP	B	34	31.665	-8.615	27.367	1.00	9.19	C
	ATOM	2321	CG	ASP	B	34	31.881	-10.030	27.892	1.00	11.22	C
	ATOM	2322	OD1	ASP	B	34	33.013	-10.576	27.882	1.00	10.96	O
60	ATOM	2323	OD2	ASP	B	34	30.916	-10.660	28.362	1.00	11.64	O
	ATOM	2324	C	ASP	B	34	32.306	-7.017	25.640	1.00	9.26	C
	ATOM	2325	O	ASP	B	34	31.726	-7.362	24.616	1.00	8.27	O
	ATOM	2326	N	ASN	B	35	32.465	-5.740	25.959	1.00	9.55	N

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ATOM   2328  CA  ASN B  35      31.791  -4.730  25.152  1.00  8.99           C
ATOM   2330  CB  ASN B  35      30.278  -4.762  25.446  1.00  9.72           C
ATOM   2333  CG  ASN B  35      29.970  -4.723  26.949  1.00 11.37           C
ATOM   2334  OD1 ASN B  35      29.559  -5.741  27.574  1.00 14.30           O
ATOM   2335  ND2 ASN B  35      30.186  -3.573  27.551  1.00  7.97           N
ATOM   2338  C   ASN B  35      32.351  -3.341  25.400  1.00  9.17           C
ATOM   2339  O   ASN B  35      33.129  -3.115  26.332  1.00  9.29           O
ATOM   2340  N   LEU B  36      31.917  -2.413  24.552  1.00  9.41           N
ATOM   2342  CA  LEU B  36      32.345  -1.026  24.581  1.00  9.12           C
ATOM   2344  CB  LEU B  36      33.308  -0.779  23.414  1.00  8.17           C
ATOM   2347  CG  LEU B  36      33.652   0.670  23.053  1.00  9.08           C
ATOM   2349  CD1 LEU B  36      34.294   1.387  24.199  1.00  9.44           C
ATOM   2353  CD2 LEU B  36      34.560   0.729  21.837  1.00 10.64           C
ATOM   2357  C   LEU B  36      31.099  -0.186  24.369  1.00  9.10           C
ATOM   2358  O   LEU B  36      30.382  -0.391  23.385  1.00  8.92           O
ATOM   2359  N   THR B  37      30.824   0.737  25.279  1.00  9.13           N
ATOM   2361  CA  THR B  37      29.653   1.596  25.151  1.00  8.93           C
ATOM   2363  CB  THR B  37      28.725   1.447  26.372  1.00  9.51           C
ATOM   2365  OG1 THR B  37      28.238   0.095  26.458  1.00 10.29           O
ATOM   2367  CG2 THR B  37      27.474   2.316  26.234  1.00 10.22           C
ATOM   2371  C   THR B  37      30.041   3.056  25.034  1.00  8.89           C
ATOM   2372  O   THR B  37      30.857   3.557  25.814  1.00  8.97           O
ATOM   2373  N   PHE B  38      29.450   3.724  24.042  1.00  7.97           N
ATOM   2375  CA  PHE B  38      29.584   5.161  23.853  1.00  8.58           C
ATOM   2377  CB  PHE B  38      29.827   5.456  22.386  1.00  8.85           C
ATOM   2380  CG  PHE B  38      31.134   4.951  21.847  1.00  7.28           C
ATOM   2381  CD1 PHE B  38      32.340   5.237  22.482  1.00  7.91           C
ATOM   2383  CE1 PHE B  38      33.544   4.811  21.942  1.00  8.72           C
ATOM   2385  CZ  PHE B  38      33.555   4.102  20.756  1.00 11.58           C
ATOM   2387  CE2 PHE B  38      32.366   3.817  20.120  1.00  9.53           C
ATOM   2389  CD2 PHE B  38      31.163   4.243  20.661  1.00  7.95           C
ATOM   2391  C   PHE B  38      28.269   5.844  24.273  1.00  8.73           C
ATOM   2392  O   PHE B  38      27.216   5.431  23.811  1.00  9.98           O
ATOM   2393  N   PRO B  39      28.293   6.842  25.163  1.00  8.76           N
ATOM   2394  CA  PRO B  39      27.036   7.460  25.636  1.00  8.86           C
ATOM   2396  CB  PRO B  39      27.497   8.386  26.780  1.00  9.34           C
ATOM   2399  CG  PRO B  39      28.785   7.866  27.199  1.00  9.35           C
ATOM   2402  CD  PRO B  39      29.448   7.348  25.916  1.00  9.58           C
ATOM   2405  C   PRO B  39      26.209   8.230  24.627  1.00  9.13           C
ATOM   2406  O   PRO B  39      24.991   8.328  24.796  1.00  8.44           O
ATOM   2407  N   LYS B  40      26.834   8.794  23.602  1.00  9.49           N
ATOM   2409  CA  LYS B  40      26.061   9.548  22.618  1.00  9.43           C
ATOM   2411  CB  LYS B  40      25.784  10.967  23.094  1.00 10.36           C
ATOM   2414  CG  LYS B  40      24.760  11.685  22.232  1.00 12.43           C
ATOM   2417  CD  LYS B  40      24.661  13.182  22.550  1.00 16.76           C
ATOM   2420  CE  LYS B  40      24.030  13.456  23.916  1.00 22.56           C
ATOM   2423  NZ  LYS B  40      24.148  14.904  24.336  1.00 28.45           N
ATOM   2427  C   LYS B  40      26.748   9.529  21.265  1.00  9.14           C
ATOM   2428  O   LYS B  40      27.597  10.355  20.962  1.00  9.57           O
ATOM   2429  N   VAL B  41      26.393   8.544  20.458  1.00  9.12           N
ATOM   2431  CA  VAL B  41      26.969   8.438  19.131  1.00  9.08           C
ATOM   2433  CB  VAL B  41      26.967   6.970  18.603  1.00  9.12           C
ATOM   2435  CG1 VAL B  41      27.769   6.059  19.553  1.00  8.74           C
ATOM   2439  CG2 VAL B  41      25.556   6.453  18.410  1.00  8.61           C
ATOM   2443  C   VAL B  41      26.243   9.323  18.136  1.00  9.87           C
ATOM   2444  O   VAL B  41      25.107   9.759  18.350  1.00  9.30           O
ATOM   2445  N   LEU B  42      26.907   9.582  17.022  1.00 10.23           N
ATOM   2447  CA  LEU B  42      26.261  10.288  15.932  1.00 11.90           C
ATOM   2449  CB  LEU B  42      27.303  10.820  14.948  1.00 11.82           C
ATOM   2452  CG  LEU B  42      28.246  11.835  15.604  1.00 13.37           C
ATOM   2454  CD1 LEU B  42      29.484  12.076  14.765  1.00 17.49           C
ATOM   2458  CD2 LEU B  42      27.504  13.150  15.923  1.00 13.74           C
ATOM   2462  C   LEU B  42      25.303   9.312  15.257  1.00 13.53           C
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	ATOM	2463	O	LEU	B	42	25.559	8.103	15.208	1.00	13.30	O
	ATOM	2464	N	THR	B	43	24.203	9.824	14.717	1.00	16.10	N
	ATOM	2466	CA	THR	B	43	23.223	8.949	14.082	1.00	17.45	C
	ATOM	2468	CB	THR	B	43	21.953	8.828	14.935	1.00	17.82	C
5	ATOM	2470	OG1	THR	B	43	21.431	10.123	15.252	1.00	20.64	O
	ATOM	2472	CG2	THR	B	43	22.241	8.193	16.296	1.00	18.56	C
	ATOM	2476	C	THR	B	43	22.842	9.433	12.691	1.00	18.34	C
	ATOM	2477	O	THR	B	43	21.866	8.947	12.117	1.00	18.70	O
	ATOM	2478	N	ASP	B	44	23.614	10.367	12.148	1.00	18.36	N
10	ATOM	2480	CA	ASP	B	44	23.349	10.867	10.776	1.00	19.37	C
	ATOM	2482	CB	ASP	B	44	24.158	12.130	10.514	1.00	18.75	C
	ATOM	2485	CG	ASP	B	44	25.649	11.906	10.660	1.00	20.65	C
	ATOM	2486	OD1	ASP	B	44	26.435	12.599	9.975	1.00	22.24	O
	ATOM	2487	OD2	ASP	B	44	26.119	11.081	11.464	1.00	18.51	O
15	ATOM	2488	C	ASP	B	44	23.632	9.875	9.640	1.00	20.76	C
	ATOM	2489	O	ASP	B	44	23.120	10.045	8.516	1.00	20.73	O
	ATOM	2490	N	LYS	B	45	24.451	8.858	9.910	1.00	22.30	N
	ATOM	2492	CA	LYS	B	45	24.845	7.850	8.930	1.00	22.65	C
	ATOM	2494	CB	LYS	B	45	26.271	8.089	8.416	1.00	23.02	C
20	ATOM	2497	CG	LYS	B	45	26.515	9.467	7.807	1.00	25.19	C
	ATOM	2500	CD	LYS	B	45	27.968	9.681	7.419	1.00	29.05	C
	ATOM	2503	CE	LYS	B	45	28.158	10.993	6.672	1.00	32.51	C
	ATOM	2506	NZ	LYS	B	45	29.514	11.592	6.878	1.00	36.79	N
	ATOM	2510	C	LYS	B	45	24.790	6.500	9.640	1.00	23.32	C
25	ATOM	2511	O	LYS	B	45	24.775	6.441	10.878	1.00	23.05	O
	ATOM	2512	N	LYS	B	46	24.771	5.425	8.856	1.00	24.20	N
	ATOM	2514	CA	LYS	B	46	24.712	4.075	9.398	1.00	23.77	C
	ATOM	2516	CB	LYS	B	46	24.033	3.132	8.406	1.00	23.95	C
	ATOM	2519	CG	LYS	B	46	22.685	3.632	7.903	1.00	24.89	C
30	ATOM	2522	CD	LYS	B	46	22.803	4.957	7.139	1.00	24.88	C
	ATOM	2525	CE	LYS	B	46	23.693	4.858	5.919	1.00	24.51	C
	ATOM	2528	NZ	LYS	B	46	24.316	6.162	5.572	1.00	20.23	N
	ATOM	2532	C	LYS	B	46	26.127	3.598	9.655	1.00	22.13	C
	ATOM	2533	O	LYS	B	46	26.717	2.916	8.828	1.00	23.19	O
35	ATOM	2534	N	TYR	B	47	26.678	3.932	10.814	1.00	21.82	N
	ATOM	2536	CA	TYR	B	47	28.065	3.580	11.084	1.00	17.30	C
	ATOM	2538	CB	TYR	B	47	28.630	4.461	12.199	1.00	16.73	C
	ATOM	2541	CG	TYR	B	47	28.584	5.926	11.880	1.00	14.44	C
	ATOM	2542	CD1	TYR	B	47	29.504	6.494	11.022	1.00	14.82	C
40	ATOM	2544	CE1	TYR	B	47	29.475	7.842	10.736	1.00	15.44	C
	ATOM	2546	CZ	TYR	B	47	28.502	8.629	11.305	1.00	14.96	C
	ATOM	2547	OH	TYR	B	47	28.457	9.966	11.010	1.00	16.74	O
	ATOM	2549	CE2	TYR	B	47	27.572	8.078	12.165	1.00	15.51	C
	ATOM	2551	CD2	TYR	B	47	27.616	6.748	12.446	1.00	15.33	C
45	ATOM	2553	C	TYR	B	47	28.239	2.119	11.467	1.00	15.49	C
	ATOM	2554	O	TYR	B	47	27.349	1.501	12.055	1.00	15.48	O
	ATOM	2555	N	SER	B	48	29.376	1.550	11.086	1.00	12.97	N
	ATOM	2557	CA	SER	B	48	29.731	0.219	11.553	1.00	12.31	C
	ATOM	2559	CB	SER	B	48	29.852	-0.787	10.419	1.00	13.21	C
50	ATOM	2562	OG	SER	B	48	30.688	-0.272	9.425	1.00	17.27	O
	ATOM	2564	C	SER	B	48	31.064	0.343	12.277	1.00	10.66	C
	ATOM	2565	O	SER	B	48	31.687	1.401	12.295	1.00	9.38	O
	ATOM	2566	N	TYR	B	49	31.478	-0.754	12.890	1.00	9.05	N
	ATOM	2568	CA	TYR	B	49	32.700	-0.772	13.663	1.00	8.64	C
55	ATOM	2570	CB	TYR	B	49	32.345	-0.790	15.142	1.00	8.24	C
	ATOM	2573	CG	TYR	B	49	31.547	0.419	15.574	1.00	7.93	C
	ATOM	2574	CD1	TYR	B	49	30.173	0.348	15.704	1.00	9.31	C
	ATOM	2576	CE1	TYR	B	49	29.444	1.443	16.089	1.00	10.10	C
	ATOM	2578	CZ	TYR	B	49	30.076	2.635	16.361	1.00	9.43	C
60	ATOM	2579	OH	TYR	B	49	29.321	3.726	16.755	1.00	11.22	O
	ATOM	2581	CE2	TYR	B	49	31.442	2.741	16.222	1.00	7.22	C
	ATOM	2583	CD2	TYR	B	49	32.169	1.629	15.833	1.00	7.22	C
	ATOM	2585	C	TYR	B	49	33.542	-1.997	13.338	1.00	8.84	C

ATOM	2586	O	TYR	B	49	33.035	-3.120	13.301	1.00	8.75	O
ATOM	2587	N	ARG	B	50	34.838	-1.764	13.149	1.00	8.80	N
ATOM	2589	CA	ARG	B	50	35.811	-2.821	12.915	1.00	8.50	C
ATOM	2591	CB	ARG	B	50	36.725	-2.427	11.771	1.00	8.52	C
ATOM	2594	CG	ARG	B	50	37.615	-3.545	11.308	1.00	9.29	C
ATOM	2597	CD	ARG	B	50	38.349	-3.220	10.048	1.00	9.53	C
ATOM	2600	NE	ARG	B	50	39.382	-2.205	10.191	1.00	8.80	N
ATOM	2602	CZ	ARG	B	50	40.631	-2.476	10.566	1.00	11.23	C
ATOM	2603	NH1	ARG	B	50	40.986	-3.721	10.901	1.00	11.51	N
ATOM	2606	NH2	ARG	B	50	41.533	-1.506	10.650	1.00	13.88	N
ATOM	2609	C	ARG	B	50	36.627	-2.987	14.186	1.00	8.77	C
ATOM	2610	O	ARG	B	50	37.063	-2.001	14.787	1.00	8.89	O
ATOM	2611	N	VAL	B	51	36.797	-4.230	14.609	1.00	9.25	N
ATOM	2613	CA	VAL	B	51	37.467	-4.543	15.860	1.00	9.05	C
ATOM	2615	CB	VAL	B	51	36.503	-5.312	16.778	1.00	9.51	C
ATOM	2617	CG1	VAL	B	51	37.193	-5.808	18.029	1.00	10.94	C
ATOM	2621	CG2	VAL	B	51	35.356	-4.425	17.179	1.00	9.48	C
ATOM	2625	C	VAL	B	51	38.729	-5.378	15.614	1.00	9.31	C
ATOM	2626	O	VAL	B	51	38.692	-6.344	14.865	1.00	9.58	O
ATOM	2627	N	VAL	B	52	39.827	-5.004	16.274	1.00	8.69	N
ATOM	2629	CA	VAL	B	52	41.136	-5.638	16.112	1.00	8.61	C
ATOM	2631	CB	VAL	B	52	42.132	-4.664	15.406	1.00	8.82	C
ATOM	2633	CG1	VAL	B	52	43.432	-5.350	15.057	1.00	8.22	C
ATOM	2637	CG2	VAL	B	52	41.503	-4.031	14.166	1.00	8.20	C
ATOM	2641	C	VAL	B	52	41.680	-6.010	17.490	1.00	8.96	C
ATOM	2642	O	VAL	B	52	41.759	-5.166	18.367	1.00	9.37	O
ATOM	2643	N	VAL	B	53	42.050	-7.276	17.677	1.00	8.28	N
ATOM	2645	CA	VAL	B	53	42.521	-7.758	18.973	1.00	9.11	C
ATOM	2647	CB	VAL	B	53	41.645	-8.918	19.482	1.00	9.88	C
ATOM	2649	CG1	VAL	B	53	42.248	-9.531	20.746	1.00	10.11	C
ATOM	2653	CG2	VAL	B	53	40.207	-8.420	19.731	1.00	10.75	C
ATOM	2657	C	VAL	B	53	43.965	-8.214	18.850	1.00	9.11	C
ATOM	2658	O	VAL	B	53	44.254	-9.170	18.105	1.00	9.06	O
ATOM	2659	N	ASN	B	54	44.873	-7.543	19.563	1.00	8.85	N
ATOM	2661	CA	ASN	B	54	46.310	-7.827	19.435	1.00	9.61	C
ATOM	2663	CB	ASN	B	54	46.676	-9.140	20.109	1.00	10.03	C
ATOM	2666	CG	ASN	B	54	47.031	-8.996	21.586	1.00	11.16	C
ATOM	2667	OD1	ASN	B	54	47.247	-10.014	22.267	1.00	16.89	O
ATOM	2668	ND2	ASN	B	54	47.126	-7.781	22.076	1.00	9.72	N
ATOM	2671	C	ASN	B	54	46.747	-7.870	17.956	1.00	10.26	C
ATOM	2672	O	ASN	B	54	47.522	-8.745	17.548	1.00	11.02	O
ATOM	2673	N	GLY	B	55	46.238	-6.928	17.168	1.00	10.54	N
ATOM	2675	CA	GLY	B	55	46.575	-6.793	15.760	1.00	9.95	C
ATOM	2678	C	GLY	B	55	45.844	-7.707	14.792	1.00	9.84	C
ATOM	2679	O	GLY	B	55	45.998	-7.522	13.579	1.00	9.89	O
ATOM	2680	N	SER	B	56	45.036	-8.629	15.310	1.00	9.14	N
ATOM	2682	CA	SER	B	56	44.226	-9.538	14.506	1.00	9.52	C
ATOM	2684	CB	SER	B	56	44.022	-10.867	15.235	1.00	10.16	C
ATOM	2687	OG	SER	B	56	43.162	-11.730	14.503	1.00	10.98	O
ATOM	2689	C	SER	B	56	42.858	-8.888	14.232	1.00	9.17	C
ATOM	2690	O	SER	B	56	42.065	-8.653	15.148	1.00	8.64	O
ATOM	2691	N	ASP	B	57	42.613	-8.558	12.976	1.00	9.04	N
ATOM	2693	CA	ASP	B	57	41.358	-7.950	12.530	1.00	8.46	C
ATOM	2695	CB	ASP	B	57	41.559	-7.526	11.067	1.00	8.57	C
ATOM	2698	CG	ASP	B	57	40.364	-6.842	10.457	1.00	9.39	C
ATOM	2699	OD1	ASP	B	57	40.383	-6.708	9.193	1.00	9.52	O
ATOM	2700	OD2	ASP	B	57	39.385	-6.414	11.106	1.00	9.56	O
ATOM	2701	C	ASP	B	57	40.201	-8.950	12.628	1.00	8.72	C
ATOM	2702	O	ASP	B	57	40.218	-10.003	11.966	1.00	8.92	O
ATOM	2703	N	LEU	B	58	39.217	-8.665	13.478	1.00	9.29	N
ATOM	2705	CA	LEU	B	58	38.021	-9.508	13.542	1.00	9.33	C
ATOM	2707	CB	LEU	B	58	37.508	-9.582	14.977	1.00	9.53	C
ATOM	2710	CG	LEU	B	58	38.564	-9.973	16.005	1.00	9.47	C

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ATOM   2712  CD1 LEU B  58      37.925 -10.156  17.379  1.00 11.17           C
ATOM   2716  CD2 LEU B  58      39.325 -11.242  15.604  1.00 11.86           C
ATOM   2720  C   LEU B  58      36.897  -9.037  12.608  1.00  9.41           C
ATOM   2721  O   LEU B  58      35.797  -9.607  12.608  1.00 10.79           O
ATOM   2722  N   GLY B  59      37.166  -8.006  11.826  1.00  9.53           N
ATOM   2724  CA  GLY B  59      36.245  -7.517  10.815  1.00  9.73           C
ATOM   2727  C   GLY B  59      35.209  -6.570  11.375  1.00  9.94           C
ATOM   2728  O   GLY B  59      35.355  -6.046  12.482  1.00  9.32           O
ATOM   2729  N   VAL B  60      34.133  -6.407  10.614  1.00 10.86           N
ATOM   2731  CA  VAL B  60      33.165  -5.356  10.840  1.00 11.57           C
ATOM   2733  CB  VAL B  60      33.048  -4.476   9.571  1.00 11.80           C
ATOM   2735  CG1 VAL B  60      32.330  -3.170   9.845  1.00 13.91           C
ATOM   2739  CG2 VAL B  60      32.361  -5.227   8.443  1.00 13.46           C
ATOM   2743  C   VAL B  60      31.806  -5.894  11.230  1.00 11.41           C
ATOM   2744  O   VAL B  60      31.409  -7.016  10.849  1.00 11.80           O
ATOM   2745  N   GLU B  61      31.090  -5.084  11.998  1.00 10.41           N
ATOM   2747  CA  GLU B  61      29.728  -5.416  12.391  1.00 11.68           C
ATOM   2749  CB  GLU B  61      29.701  -6.352  13.600  1.00 11.99           C
ATOM   2752  CG  GLU B  61      28.316  -6.803  14.051  1.00 16.37           C
ATOM   2755  CD  GLU B  61      27.469  -7.353  12.931  1.00 18.62           C
ATOM   2756  OE1 GLU B  61      26.499  -6.673  12.533  1.00 19.03           O
ATOM   2757  OE2 GLU B  61      27.791  -8.452  12.418  1.00 20.11           O
ATOM   2758  C   GLU B  61      28.994  -4.105  12.643  1.00 12.02           C
ATOM   2759  O   GLU B  61      29.616  -3.087  12.944  1.00 10.24           O
ATOM   2760  N   SER B  62      27.673  -4.129  12.540  1.00 13.34           N
ATOM   2762  CA  SER B  62      26.908  -2.919  12.741  1.00 13.52           C
ATOM   2764  CB  SER B  62      26.526  -2.289  11.407  1.00 14.00           C
ATOM   2767  OG  SER B  62      25.717  -3.176  10.647  1.00 13.96           O
ATOM   2769  C   SER B  62      25.631  -3.142  13.513  1.00 13.46           C
ATOM   2770  O   SER B  62      24.940  -2.159  13.807  1.00 14.34           O
ATOM   2771  N   ASN B  63      25.333  -4.389  13.869  1.00 12.74           N
ATOM   2773  CA  ASN B  63      24.037  -4.692  14.474  1.00 14.23           C
ATOM   2775  CB  ASN B  63      23.557  -6.123  14.156  1.00 15.00           C
ATOM   2778  CG  ASN B  63      22.193  -6.441  14.798  1.00 16.84           C
ATOM   2779  OD1 ASN B  63      21.441  -5.531  15.129  1.00 21.06           O
ATOM   2780  ND2 ASN B  63      21.895  -7.727  15.010  1.00 21.14           N
ATOM   2783  C   ASN B  63      24.089  -4.438  15.971  1.00 13.99           C
ATOM   2784  O   ASN B  63      24.093  -5.385  16.770  1.00 13.96           O
ATOM   2785  N   PHE B  64      24.126  -3.143  16.308  1.00 13.92           N
ATOM   2787  CA  PHE B  64      24.126  -2.622  17.673  1.00 12.95           C
ATOM   2789  CB  PHE B  64      25.518  -2.121  18.080  1.00 12.24           C
ATOM   2792  CG  PHE B  64      26.621  -3.056  17.698  1.00 10.08           C
ATOM   2793  CD1 PHE B  64      26.707  -4.313  18.267  1.00  9.82           C
ATOM   2795  CE1 PHE B  64      27.717  -5.176  17.900  1.00 10.20           C
ATOM   2797  CZ  PHE B  64      28.622  -4.784  16.947  1.00 10.37           C
ATOM   2799  CE2 PHE B  64      28.533  -3.542  16.383  1.00 10.15           C
ATOM   2801  CD2 PHE B  64      27.547  -2.689  16.752  1.00  9.91           C
ATOM   2803  C   PHE B  64      23.135  -1.462  17.777  1.00 12.85           C
ATOM   2804  O   PHE B  64      23.282  -0.433  17.128  1.00 12.07           O
ATOM   2805  N   ALA B  65      22.124  -1.637  18.613  1.00 13.52           N
ATOM   2807  CA  ALA B  65      21.038  -0.677  18.719  1.00 13.11           C
ATOM   2809  CB  ALA B  65      19.990  -1.165  19.701  1.00 13.88           C
ATOM   2813  C   ALA B  65      21.554   0.641  19.215  1.00 13.59           C
ATOM   2814  O   ALA B  65      22.471   0.668  20.026  1.00 13.52           O
ATOM   2815  N   VAL B  66      20.985   1.727  18.716  1.00 13.44           N
ATOM   2817  CA  VAL B  66      21.223   3.015  19.337  1.00 13.23           C
ATOM   2819  CB  VAL B  66      21.412   4.126  18.322  1.00 13.46           C
ATOM   2821  CG1 VAL B  66      21.554   5.453  19.053  1.00 14.25           C
ATOM   2825  CG2 VAL B  66      22.634   3.839  17.457  1.00 12.99           C
ATOM   2829  C   VAL B  66      20.007   3.276  20.232  1.00 13.98           C
ATOM   2830  O   VAL B  66      18.860   3.239  19.765  1.00 14.47           O
ATOM   2831  N   THR B  67      20.241   3.517  21.512  1.00 13.83           N
ATOM   2833  CA  THR B  67      19.134   3.762  22.438  1.00 15.29           C
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ATOM	2835	CB	THR	B	67	19.577	3.436	23.873	1.00	15.38	C
ATOM	2837	OG1	THR	B	67	20.710	4.246	24.225	1.00	13.23	O
ATOM	2839	CG2	THR	B	67	20.111	2.014	23.990	1.00	15.32	C
ATOM	2843	C	THR	B	67	18.644	5.220	22.269	1.00	16.76	C
ATOM	2844	O	THR	B	67	19.300	6.037	21.642	1.00	16.95	O
ATOM	2845	N	PRO	B	68	17.459	5.547	22.766	1.00	20.60	N
ATOM	2846	CA	PRO	B	68	16.921	6.907	22.635	1.00	20.58	C
ATOM	2848	CB	PRO	B	68	15.605	6.822	23.400	1.00	21.01	C
ATOM	2851	CG	PRO	B	68	15.218	5.414	23.278	1.00	21.24	C
ATOM	2854	CD	PRO	B	68	16.507	4.646	23.423	1.00	19.89	C
ATOM	2857	C	PRO	B	68	17.814	8.029	23.188	1.00	21.33	C
ATOM	2858	O	PRO	B	68	17.759	9.162	22.687	1.00	21.25	O
ATOM	2859	N	SER	B	69	18.616	7.701	24.199	1.00	22.75	N
ATOM	2861	CA	SER	B	69	19.587	8.608	24.826	1.00	21.20	C
ATOM	2863	CB	SER	B	69	20.082	7.995	26.138	1.00	21.30	C
ATOM	2866	OG	SER	B	69	20.644	6.705	25.953	1.00	22.43	O
ATOM	2868	C	SER	B	69	20.769	8.877	23.909	1.00	20.23	C
ATOM	2869	O	SER	B	69	21.553	9.820	24.111	1.00	20.71	O
ATOM	2870	N	GLY	B	70	20.897	8.016	22.908	1.00	18.48	N
ATOM	2872	CA	GLY	B	70	21.923	8.132	21.904	1.00	15.60	C
ATOM	2875	C	GLY	B	70	23.049	7.151	22.179	1.00	13.15	C
ATOM	2876	O	GLY	B	70	24.061	7.186	21.524	1.00	12.69	O
ATOM	2877	N	GLY	B	71	22.876	6.276	23.162	1.00	10.24	N
ATOM	2879	CA	GLY	B	71	23.942	5.354	23.525	1.00	10.32	C
ATOM	2882	C	GLY	B	71	24.044	4.180	22.570	1.00	10.01	C
ATOM	2883	O	GLY	B	71	23.067	3.830	21.893	1.00	9.06	O
ATOM	2884	N	GLN	B	72	25.221	3.567	22.513	1.00	9.21	N
ATOM	2886	CA	GLN	B	72	25.427	2.402	21.654	1.00	9.07	C
ATOM	2888	CB	GLN	B	72	25.841	2.818	20.242	1.00	9.26	C
ATOM	2891	CG	GLN	B	72	25.762	1.671	19.241	1.00	10.65	C
ATOM	2894	CD	GLN	B	72	25.989	2.078	17.779	1.00	11.82	C
ATOM	2895	OE1	GLN	B	72	25.420	1.465	16.838	1.00	14.64	O
ATOM	2896	NE2	GLN	B	72	26.832	3.043	17.578	1.00	8.39	N
ATOM	2899	C	GLN	B	72	26.482	1.500	22.251	1.00	8.92	C
ATOM	2900	O	GLN	B	72	27.562	1.958	22.606	1.00	9.57	O
ATOM	2901	N	THR	B	73	26.154	0.216	22.363	1.00	8.86	N
ATOM	2903	CA	THR	B	73	27.059	-0.781	22.906	1.00	9.09	C
ATOM	2905	CB	THR	B	73	26.344	-1.572	24.005	1.00	9.39	C
ATOM	2907	OG1	THR	B	73	25.995	-0.692	25.072	1.00	9.52	O
ATOM	2909	CG2	THR	B	73	27.270	-2.612	24.627	1.00	10.72	C
ATOM	2913	C	THR	B	73	27.509	-1.748	21.809	1.00	9.41	C
ATOM	2914	O	THR	B	73	26.680	-2.437	21.200	1.00	10.31	O
ATOM	2915	N	ILE	B	74	28.812	-1.757	21.563	1.00	9.66	N
ATOM	2917	CA	ILE	B	74	29.458	-2.662	20.630	1.00	9.26	C
ATOM	2919	CB	ILE	B	74	30.716	-2.000	20.065	1.00	9.30	C
ATOM	2921	CG1	ILE	B	74	30.322	-0.764	19.234	1.00	10.63	C
ATOM	2924	CD1	ILE	B	74	31.405	0.279	19.094	1.00	13.52	C
ATOM	2928	CG2	ILE	B	74	31.494	-2.996	19.212	1.00	9.71	C
ATOM	2932	C	ILE	B	74	29.779	-3.915	21.441	1.00	8.04	C
ATOM	2933	O	ILE	B	74	30.553	-3.873	22.389	1.00	8.84	O
ATOM	2934	N	ASN	B	75	29.117	-5.012	21.103	1.00	8.10	N
ATOM	2936	CA	ASN	B	75	29.257	-6.266	21.819	1.00	7.89	C
ATOM	2938	CB	ASN	B	75	27.908	-6.975	21.781	1.00	8.13	C
ATOM	2941	CG	ASN	B	75	27.942	-8.323	22.426	1.00	8.20	C
ATOM	2942	OD1	ASN	B	75	28.856	-8.662	23.195	1.00	7.74	O
ATOM	2943	ND2	ASN	B	75	26.946	-9.120	22.108	1.00	11.67	N
ATOM	2946	C	ASN	B	75	30.324	-7.129	21.149	1.00	8.35	C
ATOM	2947	O	ASN	B	75	30.128	-7.635	20.029	1.00	7.57	O
ATOM	2948	N	PHE	B	76	31.449	-7.309	21.831	1.00	8.93	N
ATOM	2950	CA	PHE	B	76	32.572	-8.022	21.228	1.00	9.24	C
ATOM	2952	CB	PHE	B	76	33.846	-7.826	22.054	1.00	9.38	C
ATOM	2955	CG	PHE	B	76	34.319	-6.391	22.076	1.00	9.36	C
ATOM	2956	CD1	PHE	B	76	34.307	-5.628	20.921	1.00	10.05	C

	ATOM	2958	CE1	PHE	B	76	34.715	-4.318	20.939	1.00	9.26	C
	ATOM	2960	CZ	PHE	B	76	35.131	-3.763	22.112	1.00	8.99	C
	ATOM	2962	CE2	PHE	B	76	35.143	-4.500	23.259	1.00	10.57	C
	ATOM	2964	CD2	PHE	B	76	34.745	-5.806	23.246	1.00	10.62	C
5	ATOM	2966	C	PHE	B	76	32.260	-9.502	20.999	1.00	9.01	C
	ATOM	2967	O	PHE	B	76	32.893	-10.149	20.169	1.00	9.14	O
	ATOM	2968	N	LEU	B	77	31.268	-10.038	21.708	1.00	9.74	N
	ATOM	2970	CA	LEU	B	77	30.894	-11.444	21.525	1.00	9.62	C
	ATOM	2972	CB	LEU	B	77	29.844	-11.840	22.565	1.00	9.60	C
10	ATOM	2975	CG	LEU	B	77	30.361	-12.157	23.981	1.00	11.85	C
	ATOM	2977	CD1	LEU	B	77	31.102	-11.029	24.640	1.00	13.22	C
	ATOM	2981	CD2	LEU	B	77	29.174	-12.593	24.854	1.00	12.68	C
	ATOM	2985	C	LEU	B	77	30.400	-11.713	20.077	1.00	9.32	C
	ATOM	2986	O	LEU	B	77	30.481	-12.843	19.574	1.00	10.46	O
15	ATOM	2987	N	GLN	B	78	29.907	-10.671	19.415	1.00	9.10	N
	ATOM	2989	CA	GLN	B	78	29.450	-10.748	18.032	1.00	10.10	C
	ATOM	2991	CB	GLN	B	78	28.517	-9.569	17.697	1.00	10.43	C
	ATOM	2994	CG	GLN	B	78	27.143	-9.734	18.379	1.00	9.66	C
	ATOM	2997	CD	GLN	B	78	26.224	-8.538	18.295	1.00	11.71	C
20	ATOM	2998	OE1	GLN	B	78	25.803	-8.019	19.329	1.00	11.16	O
	ATOM	2999	NE2	GLN	B	78	25.871	-8.113	17.071	1.00	13.17	N
	ATOM	3002	C	GLN	B	78	30.612	-10.816	17.042	1.00	11.05	C
	ATOM	3003	O	GLN	B	78	30.379	-11.128	15.859	1.00	13.23	O
	ATOM	3004	N	TYR	B	79	31.826	-10.506	17.500	1.00	10.96	N
25	ATOM	3006	CA	TYR	B	79	33.026	-10.570	16.654	1.00	12.02	C
	ATOM	3008	CB	TYR	B	79	33.915	-9.339	16.842	1.00	11.81	C
	ATOM	3011	CG	TYR	B	79	33.345	-8.005	16.481	1.00	10.03	C
	ATOM	3012	CD1	TYR	B	79	33.681	-7.372	15.280	1.00	8.25	C
	ATOM	3014	CE1	TYR	B	79	33.169	-6.116	14.965	1.00	8.39	C
30	ATOM	3016	CZ	TYR	B	79	32.322	-5.483	15.842	1.00	8.21	C
	ATOM	3017	OH	TYR	B	79	31.827	-4.250	15.505	1.00	8.87	O
	ATOM	3019	CE2	TYR	B	79	31.972	-6.104	17.033	1.00	10.02	C
	ATOM	3021	CD2	TYR	B	79	32.487	-7.347	17.345	1.00	9.93	C
	ATOM	3023	C	TYR	B	79	33.940	-11.728	16.985	1.00	13.56	C
35	ATOM	3024	O	TYR	B	79	34.689	-12.190	16.120	1.00	13.49	O
	ATOM	3025	N	ASN	B	80	33.919	-12.162	18.242	1.00	15.17	N
	ATOM	3027	CA	ASN	B	80	34.925	-13.076	18.751	1.00	14.48	C
	ATOM	3029	CB	ASN	B	80	35.582	-12.444	19.978	1.00	14.14	C
	ATOM	3032	CG	ASN	B	80	36.890	-13.105	20.361	1.00	14.62	C
40	ATOM	3033	OD1	ASN	B	80	37.651	-13.558	19.509	1.00	16.67	O
	ATOM	3034	ND2	ASN	B	80	37.153	-13.163	21.664	1.00	13.88	N
	ATOM	3037	C	ASN	B	80	34.352	-14.437	19.084	1.00	14.66	C
	ATOM	3038	O	ASN	B	80	34.712	-15.056	20.083	1.00	13.88	O
	ATOM	3039	N	LYS	B	81	33.412	-14.879	18.257	1.00	15.51	N
45	ATOM	3041	CA	LYS	B	81	32.859	-16.233	18.368	1.00	16.46	C
	ATOM	3043	CB	LYS	B	81	33.943	-17.263	17.992	1.00	16.91	C
	ATOM	3046	CG	LYS	B	81	34.644	-16.962	16.663	1.00	20.41	C
	ATOM	3049	CD	LYS	B	81	35.616	-18.076	16.175	1.00	26.52	C
	ATOM	3052	CE	LYS	B	81	36.392	-18.798	17.293	1.00	31.18	C
50	ATOM	3055	NZ	LYS	B	81	37.525	-19.720	16.822	1.00	38.55	N
	ATOM	3059	C	LYS	B	81	32.216	-16.559	19.735	1.00	15.91	C
	ATOM	3060	O	LYS	B	81	32.322	-17.678	20.227	1.00	16.16	O
	ATOM	3061	N	GLY	B	82	31.522	-15.575	20.307	1.00	15.03	N
	ATOM	3063	CA	GLY	B	82	30.735	-15.736	21.512	1.00	15.26	C
55	ATOM	3066	C	GLY	B	82	31.492	-15.553	22.800	1.00	15.69	C
	ATOM	3067	O	GLY	B	82	30.960	-15.833	23.857	1.00	15.34	O
	ATOM	3068	N	TYR	B	83	32.721	-15.052	22.708	1.00	16.90	N
	ATOM	3070	CA	TYR	B	83	33.550	-14.806	23.873	1.00	17.19	C
	ATOM	3072	CB	TYR	B	83	34.815	-15.667	23.808	1.00	18.20	C
60	ATOM	3075	CG	TYR	B	83	34.583	-17.155	23.934	1.00	23.38	C
	ATOM	3076	CD1	TYR	B	83	34.448	-17.745	25.181	1.00	28.89	C
	ATOM	3078	CE1	TYR	B	83	34.239	-19.105	25.310	1.00	31.05	C
	ATOM	3080	CZ	TYR	B	83	34.170	-19.896	24.184	1.00	32.68	C

ATOM	3081	OH	TYR	B	83	33.962	-21.259	24.329	1.00	36.36	O
ATOM	3083	CE2	TYR	B	83	34.305	-19.340	22.930	1.00	30.78	C
ATOM	3085	CD2	TYR	B	83	34.506	-17.972	22.810	1.00	28.62	C
ATOM	3087	C	TYR	B	83	34.006	-13.355	23.925	1.00	16.15	C
ATOM	3088	O	TYR	B	83	34.169	-12.715	22.894	1.00	15.94	O
ATOM	3089	N	GLY	B	84	34.190	-12.828	25.134	1.00	15.91	N
ATOM	3091	CA	GLY	B	84	34.842	-11.539	25.278	1.00	14.80	C
ATOM	3094	C	GLY	B	84	36.359	-11.675	25.047	1.00	13.96	C
ATOM	3095	O	GLY	B	84	36.882	-12.737	24.675	1.00	14.24	O
ATOM	3096	N	VAL	B	85	37.071	-10.586	25.311	1.00	11.64	N
ATOM	3098	CA	VAL	B	85	38.497	-10.478	25.027	1.00	12.19	C
ATOM	3100	CB	VAL	B	85	38.761	-9.214	24.159	1.00	11.76	C
ATOM	3102	CG1	VAL	B	85	40.254	-9.019	23.892	1.00	12.27	C
ATOM	3106	CG2	VAL	B	85	37.970	-9.236	22.852	1.00	12.61	C
ATOM	3110	C	VAL	B	85	39.258	-10.315	26.329	1.00	12.06	C
ATOM	3111	O	VAL	B	85	38.954	-9.435	27.133	1.00	11.39	O
ATOM	3112	N	ALA	B	86	40.263	-11.154	26.554	1.00	11.94	N
ATOM	3114	CA	ALA	B	86	41.077	-11.026	27.752	1.00	12.43	C
ATOM	3116	CB	ALA	B	86	42.221	-12.031	27.709	1.00	12.46	C
ATOM	3120	C	ALA	B	86	41.630	-9.606	27.895	1.00	12.58	C
ATOM	3121	O	ALA	B	86	42.145	-9.034	26.921	1.00	11.12	O
ATOM	3122	N	ASP	B	87	41.542	-9.046	29.101	1.00	12.96	N
ATOM	3124	CA	ASP	B	87	41.977	-7.664	29.319	1.00	13.26	C
ATOM	3126	CB	ASP	B	87	41.413	-7.038	30.599	1.00	12.94	C
ATOM	3129	CG	ASP	B	87	41.973	-7.621	31.863	1.00	15.55	C
ATOM	3130	OD1	ASP	B	87	42.925	-8.435	31.811	1.00	16.54	O
ATOM	3131	OD2	ASP	B	87	41.478	-7.304	32.971	1.00	17.64	O
ATOM	3132	C	ASP	B	87	43.473	-7.446	29.177	1.00	13.14	C
ATOM	3133	O	ASP	B	87	43.923	-6.303	29.211	1.00	13.88	O
ATOM	3134	N	THR	B	88	44.222	-8.529	28.986	1.00	13.35	N
ATOM	3136	CA	THR	B	88	45.648	-8.426	28.770	1.00	13.89	C
ATOM	3138	CB	THR	B	88	46.376	-9.702	29.223	1.00	13.97	C
ATOM	3140	OG1	THR	B	88	45.720	-10.853	28.692	1.00	14.23	O
ATOM	3142	CG2	THR	B	88	46.312	-9.865	30.728	1.00	15.70	C
ATOM	3146	C	THR	B	88	45.972	-8.179	27.308	1.00	14.14	C
ATOM	3147	O	THR	B	88	47.138	-7.960	26.978	1.00	16.23	O
ATOM	3148	N	LYS	B	89	44.961	-8.217	26.444	1.00	13.28	N
ATOM	3150	CA	LYS	B	89	45.163	-7.962	25.021	1.00	13.04	C
ATOM	3152	CB	LYS	B	89	44.317	-8.922	24.185	1.00	13.74	C
ATOM	3155	CG	LYS	B	89	44.531	-10.371	24.562	1.00	15.15	C
ATOM	3158	CD	LYS	B	89	43.821	-11.309	23.612	1.00	18.37	C
ATOM	3161	CE	LYS	B	89	43.980	-12.771	24.006	1.00	20.95	C
ATOM	3164	NZ	LYS	B	89	43.412	-13.640	22.933	1.00	24.82	N
ATOM	3168	C	LYS	B	89	44.798	-6.531	24.667	1.00	12.47	C
ATOM	3169	O	LYS	B	89	44.022	-5.884	25.369	1.00	12.82	O
ATOM	3170	N	THR	B	90	45.377	-6.023	23.589	1.00	11.85	N
ATOM	3172	CA	THR	B	90	45.017	-4.714	23.072	1.00	11.57	C
ATOM	3174	CB	THR	B	90	46.177	-4.184	22.273	1.00	11.83	C
ATOM	3176	OG1	THR	B	90	47.280	-3.912	23.164	1.00	14.04	O
ATOM	3178	CG2	THR	B	90	45.838	-2.872	21.626	1.00	13.14	C
ATOM	3182	C	THR	B	90	43.780	-4.827	22.180	1.00	11.03	C
ATOM	3183	O	THR	B	90	43.684	-5.748	21.355	1.00	10.60	O
ATOM	3184	N	ILE	B	91	42.839	-3.893	22.344	1.00	10.28	N
ATOM	3186	CA	ILE	B	91	41.640	-3.871	21.530	1.00	10.28	C
ATOM	3188	CB	ILE	B	91	40.361	-4.011	22.361	1.00	10.29	C
ATOM	3190	CG1	ILE	B	91	40.407	-5.261	23.238	1.00	11.66	C
ATOM	3193	CD1	ILE	B	91	39.298	-5.354	24.285	1.00	13.60	C
ATOM	3197	CG2	ILE	B	91	39.141	-4.060	21.408	1.00	10.59	C
ATOM	3201	C	ILE	B	91	41.597	-2.536	20.815	1.00	10.62	C
ATOM	3202	O	ILE	B	91	41.700	-1.489	21.442	1.00	10.66	O
ATOM	3203	N	GLN	B	92	41.490	-2.563	19.497	1.00	10.49	N
ATOM	3205	CA	GLN	B	92	41.313	-1.326	18.764	1.00	10.71	C
ATOM	3207	CB	GLN	B	92	42.410	-1.146	17.719	1.00	11.65	C

	ATOM	3210	CG	GLN	B	92	43.778	-0.942	18.282	1.00	15.30	C
	ATOM	3213	CD	GLN	B	92	44.809	-1.227	17.215	1.00	21.75	C
	ATOM	3214	OE1	GLN	B	92	45.030	-2.382	16.855	1.00	23.06	O
	ATOM	3215	NE2	GLN	B	92	45.388	-0.176	16.654	1.00	29.53	N
5	ATOM	3218	C	GLN	B	92	39.964	-1.384	18.063	1.00	9.76	C
	ATOM	3219	O	GLN	B	92	39.599	-2.416	17.499	1.00	9.52	O
	ATOM	3220	N	VAL	B	93	39.225	-0.286	18.109	1.00	8.49	N
	ATOM	3222	CA	VAL	B	93	37.922	-0.204	17.447	1.00	8.46	C
	ATOM	3224	CB	VAL	B	93	36.754	-0.031	18.452	1.00	8.40	C
10	ATOM	3226	CG1	VAL	B	93	35.408	-0.068	17.715	1.00	8.33	C
	ATOM	3230	CG2	VAL	B	93	36.818	-1.110	19.565	1.00	9.21	C
	ATOM	3234	C	VAL	B	93	37.954	0.979	16.498	1.00	8.80	C
	ATOM	3235	O	VAL	B	93	38.313	2.100	16.913	1.00	9.38	O
	ATOM	3236	N	PHE	B	94	37.585	0.735	15.235	1.00	7.89	N
15	ATOM	3238	CA	PHE	B	94	37.514	1.756	14.207	1.00	8.32	C
	ATOM	3240	CB	PHE	B	94	38.303	1.336	12.954	1.00	8.53	C
	ATOM	3243	CG	PHE	B	94	39.774	1.165	13.207	1.00	8.61	C
	ATOM	3244	CD1	PHE	B	94	40.658	2.188	12.898	1.00	10.93	C
	ATOM	3246	CE1	PHE	B	94	42.001	2.046	13.153	1.00	11.25	C
20	ATOM	3248	CZ	PHE	B	94	42.482	0.899	13.732	1.00	10.46	C
	ATOM	3250	CE2	PHE	B	94	41.644	-0.131	14.027	1.00	10.75	C
	ATOM	3252	CD2	PHE	B	94	40.273	0.003	13.776	1.00	10.71	C
	ATOM	3254	C	PHE	B	94	36.070	1.974	13.819	1.00	8.61	C
	ATOM	3255	O	PHE	B	94	35.314	1.013	13.648	1.00	9.56	O
25	ATOM	3256	N	VAL	B	95	35.687	3.231	13.647	1.00	8.45	N
	ATOM	3258	CA	VAL	B	95	34.370	3.546	13.104	1.00	9.19	C
	ATOM	3260	CB	VAL	B	95	33.767	4.834	13.699	1.00	9.11	C
	ATOM	3262	CG1	VAL	B	95	34.614	6.052	13.439	1.00	11.33	C
	ATOM	3266	CG2	VAL	B	95	32.342	5.013	13.203	1.00	10.43	C
30	ATOM	3270	C	VAL	B	95	34.535	3.607	11.594	1.00	9.06	C
	ATOM	3271	O	VAL	B	95	35.480	4.251	11.094	1.00	9.36	O
	ATOM	3272	N	VAL	B	96	33.660	2.896	10.888	1.00	10.07	N
	ATOM	3274	CA	VAL	B	96	33.731	2.760	9.435	1.00	10.75	C
	ATOM	3276	CB	VAL	B	96	33.633	1.287	9.027	1.00	11.31	C
35	ATOM	3278	CG1	VAL	B	96	33.699	1.117	7.507	1.00	12.23	C
	ATOM	3282	CG2	VAL	B	96	34.726	0.475	9.716	1.00	11.29	C
	ATOM	3286	C	VAL	B	96	32.598	3.544	8.811	1.00	11.08	C
	ATOM	3287	O	VAL	B	96	31.425	3.388	9.170	1.00	11.36	O
	ATOM	3288	N	ILE	B	97	32.948	4.403	7.866	1.00	11.39	N
40	ATOM	3290	CA	ILE	B	97	31.959	5.265	7.236	1.00	12.42	C
	ATOM	3292	CB	ILE	B	97	32.677	6.496	6.644	1.00	12.48	C
	ATOM	3294	CG1	ILE	B	97	33.614	7.145	7.677	1.00	12.52	C
	ATOM	3297	CD1	ILE	B	97	32.936	7.612	8.958	1.00	14.19	C
	ATOM	3301	CG2	ILE	B	97	31.669	7.488	6.082	1.00	12.90	C
45	ATOM	3305	C	ILE	B	97	31.234	4.501	6.130	1.00	13.56	C
	ATOM	3306	O	ILE	B	97	31.898	3.883	5.308	1.00	13.06	O
	ATOM	3307	N	PRO	B	98	29.898	4.524	6.113	1.00	15.09	N
	ATOM	3308	CA	PRO	B	98	29.132	3.809	5.086	1.00	16.07	C
	ATOM	3310	CB	PRO	B	98	27.696	3.899	5.600	1.00	15.86	C
50	ATOM	3313	CG	PRO	B	98	27.661	5.122	6.370	1.00	16.80	C
	ATOM	3316	CD	PRO	B	98	29.007	5.184	7.076	1.00	14.97	C
	ATOM	3319	C	PRO	B	98	29.267	4.451	3.718	1.00	17.70	C
	ATOM	3320	O	PRO	B	98	29.605	5.631	3.592	1.00	17.04	O
	ATOM	3321	N	ASP	B	99	29.014	3.649	2.696	1.00	20.10	N
55	ATOM	3323	CA	ASP	B	99	29.082	4.091	1.303	1.00	20.04	C
	ATOM	3325	CB	ASP	B	99	28.029	5.172	1.061	1.00	21.12	C
	ATOM	3328	CG	ASP	B	99	26.612	4.657	1.337	1.00	23.44	C
	ATOM	3329	OD1	ASP	B	99	26.291	3.537	0.874	1.00	27.92	O
	ATOM	3330	OD2	ASP	B	99	25.761	5.269	2.020	1.00	28.30	O
60	ATOM	3331	C	ASP	B	99	30.494	4.496	0.860	1.00	19.38	C
	ATOM	3332	O	ASP	B	99	30.646	5.358	-0.015	1.00	19.12	O
	ATOM	3333	N	THR	B	100	31.521	3.879	1.460	1.00	17.96	N
	ATOM	3335	CA	THR	B	100	32.917	4.061	1.030	1.00	18.27	C

ATOM	3337	CB	THR	B	100	33.757	4.812	2.091	1.00	18.29	C
ATOM	3339	OG1	THR	B	100	33.964	3.969	3.249	1.00	16.21	O
ATOM	3341	CG2	THR	B	100	33.041	6.065	2.595	1.00	18.22	C
ATOM	3345	C	THR	B	100	33.606	2.725	0.744	1.00	18.55	C
ATOM	3346	O	THR	B	100	34.839	2.644	0.724	1.00	18.17	O
ATOM	3347	N	GLY	B	101	32.813	1.676	0.537	1.00	19.93	N
ATOM	3349	CA	GLY	B	101	33.346	0.341	0.306	1.00	19.59	C
ATOM	3352	C	GLY	B	101	34.217	-0.119	1.467	1.00	19.66	C
ATOM	3353	O	GLY	B	101	35.181	-0.861	1.285	1.00	19.57	O
ATOM	3354	N	ASN	B	102	33.856	0.343	2.661	1.00	19.84	N
ATOM	3356	CA	ASN	B	102	34.580	0.071	3.906	1.00	19.99	C
ATOM	3358	CB	ASN	B	102	34.517	-1.406	4.266	1.00	20.27	C
ATOM	3361	CG	ASN	B	102	33.148	-1.823	4.683	1.00	22.54	C
ATOM	3362	OD1	ASN	B	102	32.856	-1.970	5.871	1.00	26.86	O
ATOM	3363	ND2	ASN	B	102	32.293	-2.035	3.709	1.00	25.85	N
ATOM	3366	C	ASN	B	102	36.016	0.557	3.943	1.00	19.98	C
ATOM	3367	O	ASN	B	102	36.805	0.124	4.782	1.00	19.09	O
ATOM	3368	N	SER	B	103	36.343	1.504	3.076	1.00	20.60	N
ATOM	3370	CA	SER	B	103	37.707	1.990	2.986	1.00	19.63	C
ATOM	3372	CB	SER	B	103	38.016	2.353	1.541	1.00	20.16	C
ATOM	3375	OG	SER	B	103	37.253	3.481	1.156	1.00	22.36	O
ATOM	3377	C	SER	B	103	37.979	3.214	3.870	1.00	18.01	C
ATOM	3378	O	SER	B	103	39.137	3.525	4.144	1.00	18.98	O
ATOM	3379	N	GLU	B	104	36.936	3.918	4.294	1.00	15.67	N
ATOM	3381	CA	GLU	B	104	37.135	5.116	5.120	1.00	13.43	C
ATOM	3383	CB	GLU	B	104	36.265	6.297	4.656	1.00	12.81	C
ATOM	3386	CG	GLU	B	104	36.679	7.618	5.313	1.00	13.27	C
ATOM	3389	CD	GLU	B	104	35.732	8.776	5.029	1.00	14.64	C
ATOM	3390	OE1	GLU	B	104	34.919	8.679	4.069	1.00	15.08	O
ATOM	3391	OE2	GLU	B	104	35.814	9.803	5.742	1.00	14.12	O
ATOM	3392	C	GLU	B	104	36.797	4.780	6.558	1.00	12.23	C
ATOM	3393	O	GLU	B	104	35.659	4.435	6.855	1.00	11.79	O
ATOM	3394	N	GLU	B	105	37.793	4.856	7.439	1.00	11.65	N
ATOM	3396	CA	GLU	B	105	37.573	4.534	8.845	1.00	10.60	C
ATOM	3398	CB	GLU	B	105	37.830	3.047	9.102	1.00	10.23	C
ATOM	3401	CG	GLU	B	105	39.288	2.653	8.998	1.00	11.51	C
ATOM	3404	CD	GLU	B	105	39.569	1.177	9.250	1.00	13.25	C
ATOM	3405	OE1	GLU	B	105	40.772	0.827	9.367	1.00	14.63	O
ATOM	3406	OE2	GLU	B	105	38.617	0.366	9.341	1.00	11.87	O
ATOM	3407	C	GLU	B	105	38.476	5.381	9.732	1.00	9.65	C
ATOM	3408	O	GLU	B	105	39.492	5.931	9.272	1.00	10.58	O
ATOM	3409	N	TYR	B	106	38.112	5.465	11.014	1.00	9.46	N
ATOM	3411	CA	TYR	B	106	38.842	6.260	12.004	1.00	9.17	C
ATOM	3413	CB	TYR	B	106	38.119	7.593	12.258	1.00	9.00	C
ATOM	3416	CG	TYR	B	106	37.989	8.388	10.990	1.00	9.70	C
ATOM	3417	CD1	TYR	B	106	39.000	9.227	10.565	1.00	10.02	C
ATOM	3419	CE1	TYR	B	106	38.900	9.908	9.378	1.00	11.83	C
ATOM	3421	CZ	TYR	B	106	37.797	9.755	8.584	1.00	10.67	C
ATOM	3422	OH	TYR	B	106	37.719	10.438	7.368	1.00	13.02	O
ATOM	3424	CE2	TYR	B	106	36.784	8.909	8.958	1.00	10.26	C
ATOM	3426	CD2	TYR	B	106	36.887	8.224	10.166	1.00	9.29	C
ATOM	3428	C	TYR	B	106	38.949	5.516	13.318	1.00	9.17	C
ATOM	3429	O	TYR	B	106	37.963	4.945	13.774	1.00	8.39	O
ATOM	3430	N	ILE	B	107	40.116	5.546	13.955	1.00	8.89	N
ATOM	3432	CA	ILE	B	107	40.211	4.966	15.288	1.00	8.90	C
ATOM	3434	CB	ILE	B	107	41.652	5.037	15.848	1.00	9.24	C
ATOM	3436	CG1	ILE	B	107	41.770	4.245	17.155	1.00	10.76	C
ATOM	3439	CD1	ILE	B	107	41.571	2.765	16.979	1.00	12.64	C
ATOM	3443	CG2	ILE	B	107	42.088	6.484	16.051	1.00	9.73	C
ATOM	3447	C	ILE	B	107	39.211	5.679	16.194	1.00	9.06	C
ATOM	3448	O	ILE	B	107	39.102	6.912	16.171	1.00	9.11	O
ATOM	3449	N	ILE	B	108	38.448	4.908	16.958	1.00	8.10	N
ATOM	3451	CA	ILE	B	108	37.466	5.505	17.858	1.00	8.46	C

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ATOM   3453  CB  ILE B 108      36.038   5.362  17.263  1.00  7.92           C
ATOM   3455  CG1 ILE B 108      35.058   6.318  17.933  1.00  8.44           C
ATOM   3458  CD1 ILE B 108      35.451   7.735  17.791  1.00 10.51           C
ATOM   3462  CG2 ILE B 108      35.548   3.943  17.367  1.00  7.73           C
ATOM   3466  C   ILE B 108      37.577   5.032  19.315  1.00  9.21           C
ATOM   3467  O   ILE B 108      37.028   5.675  20.206  1.00  9.94           O
ATOM   3468  N   ALA B 109      38.295   3.939  19.566  1.00  8.98           N
ATOM   3470  CA  ALA B 109      38.565   3.467  20.929  1.00  8.87           C
ATOM   3472  CB  ALA B 109      37.358   2.771  21.525  1.00  9.01           C
ATOM   3476  C   ALA B 109      39.744   2.507  20.935  1.00  8.73           C
ATOM   3477  O   ALA B 109      39.957   1.757  19.994  1.00  9.76           O
ATOM   3478  N   GLU B 110      40.533   2.576  21.998  1.00  9.54           N
ATOM   3480  CA  GLU B 110      41.607   1.634  22.215  1.00  9.46           C
ATOM   3482  CB  GLU B 110      42.946   2.257  21.824  1.00 10.74           C
ATOM   3485  CG  GLU B 110      44.120   1.290  21.888  1.00 14.55           C
ATOM   3488  CD  GLU B 110      45.414   1.938  21.419  1.00 21.68           C
ATOM   3489  OE1 GLU B 110      45.950   2.812  22.134  1.00 25.49           O
ATOM   3490  OE2 GLU B 110      45.877   1.587  20.321  1.00 27.22           O
ATOM   3491  C   GLU B 110      41.678   1.209  23.680  1.00  9.73           C
ATOM   3492  O   GLU B 110      41.720   2.056  24.582  1.00 10.15           O
ATOM   3493  N   TRP B 111      41.703  -0.098  23.894  1.00  9.39           N
ATOM   3495  CA  TRP B 111      41.990  -0.671  25.187  1.00 10.20           C
ATOM   3497  CB  TRP B 111      41.076  -1.840  25.491  1.00  9.67           C
ATOM   3500  CG  TRP B 111      41.470  -2.528  26.787  1.00  8.52           C
ATOM   3501  CD1 TRP B 111      42.308  -3.581  26.928  1.00  9.95           C
ATOM   3503  NE1 TRP B 111      42.456  -3.906  28.256  1.00 13.10           N
ATOM   3505  CE2 TRP B 111      41.728  -3.017  29.010  1.00 10.98           C
ATOM   3506  CD2 TRP B 111      41.102  -2.128  28.116  1.00  9.50           C
ATOM   3507  CE3 TRP B 111      40.282  -1.123  28.636  1.00 10.62           C
ATOM   3509  CZ3 TRP B 111      40.114  -1.040  30.002  1.00 12.26           C
ATOM   3511  CH2 TRP B 111      40.756  -1.929  30.857  1.00  9.96           C
ATOM   3513  CZ2 TRP B 111      41.561  -2.926  30.385  1.00 12.41           C
ATOM   3515  C   TRP B 111      43.423  -1.193  25.169  1.00 13.35           C
ATOM   3516  O   TRP B 111      43.775  -2.031  24.344  1.00 11.63           O
ATOM   3517  N   LYS B 112      44.263  -0.666  26.056  1.00 16.48           N
ATOM   3519  CA  LYS B 112      45.593  -1.223  26.244  1.00 20.35           C
ATOM   3521  CB  LYS B 112      46.627  -0.459  25.436  1.00 22.00           C
ATOM   3524  CG  LYS B 112      47.926  -1.228  25.265  1.00 26.13           C
ATOM   3527  CD  LYS B 112      49.024  -0.337  24.701  1.00 31.80           C
ATOM   3530  CE  LYS B 112      48.724   0.098  23.278  1.00 34.89           C
ATOM   3533  NZ  LYS B 112      49.811   0.945  22.716  1.00 40.47           N
ATOM   3537  C   LYS B 112      45.946  -1.177  27.725  1.00 22.27           C
ATOM   3538  O   LYS B 112      46.204  -0.081  28.253  1.00 23.96           O
ATOM   3539  BR  BR1 C   1      32.421  56.008  18.617  1.00  7.69           B
ATOM   3540  BR  BR1 C   2      29.535  49.785   7.652  1.00  7.89           B
ATOM   3541  BR  BR1 C   3      14.888  42.517   9.414  1.00  6.57           B
ATOM   3542  BR  BR1 C   4      25.062  15.958  16.407  1.00 10.90           B
ATOM   3543  BR  BR1 C   5      33.144  18.262   4.026  1.00 20.03           B
ATOM   3544  BR  BR1 C   6      40.800  30.559  10.185  1.00 12.36           B
ATOM   3545  BR  BR1 C   7      30.248  54.190  19.852  1.00 14.74           B
ATOM   3546  BR  BR1 C   8      38.772  41.003  24.687  1.00 22.37           B
ATOM   3547  BR  BR1 C   9      26.990   5.115  28.326  1.00 15.47           B
ATOM   3548  BR  BR1 C  10      40.148   5.267  23.548  1.00  2.00           B
ATOM   3549  BR  BR1 C  11      40.494 -13.035  23.333  1.00 14.97           B
ATOM   3550  BR  BR1 C  12      26.318 -12.293  15.448  1.00 14.38           B
ATOM   3551  BR  BR1 C  13      31.199 -18.188  15.135  1.00  9.41           B
ATOM   3552  BR  BR1 C  14      32.035 -14.040  15.742  1.00 12.63           B
ATOM   3553  BR  BR1 C  15      29.171  31.139   8.101  1.00  2.00           B
ATOM   3554  BR  BR1 C  16      28.318  -4.326   9.061  1.00  2.00           B
ATOM   3555  O   HOH D   1      45.016  -8.481  11.093  1.00 14.02           O
ATOM   3558  O   HOH D   2      39.945   9.187  15.069  1.00 13.06           O
ATOM   3561  O   HOH D   3      37.478  27.672  11.707  1.00 16.80           O
ATOM   3564  O   HOH D   4      44.772  -4.577  18.363  1.00 14.26           O
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	ATOM	3567	O	HOH D	5	28.336	-7.855	25.862	1.00	13.43		O
	ATOM	3570	O	HOH D	6	23.544	0.467	24.663	1.00	16.56		O
	ATOM	3573	O	HOH D	7	29.531	-5.991	30.538	1.00	15.82		O
	ATOM	3576	O	HOH D	8	24.673	37.622	24.477	1.00	15.51		O
5	ATOM	3579	O	HOH D	9	33.907	51.248	18.235	1.00	18.02		O
	ATOM	3582	O	HOH D	10	29.468	6.207	15.500	1.00	14.54		O
	ATOM	3585	O	HOH D	11	33.083	30.830	21.409	1.00	17.28		O
	ATOM	3588	O	HOH D	12	22.901	44.556	18.335	1.00	14.73		O
	ATOM	3591	O	HOH D	13	31.027	20.428	25.544	1.00	16.68		O
10	ATOM	3594	O	HOH D	14	30.995	8.205	30.208	1.00	19.66		O
	ATOM	3597	O	HOH D	15	28.108	-13.792	35.951	1.00	20.73		O
	ATOM	3600	O	HOH D	16	42.527	6.434	12.302	1.00	18.94		O
	ATOM	3603	O	HOH D	17	32.508	14.749	4.982	1.00	23.91		O
	ATOM	3606	O	HOH D	18	23.468	6.955	26.616	1.00	18.06		O
15	ATOM	3609	O	HOH D	19	22.712	2.972	25.766	1.00	20.00		O
	ATOM	3612	O	HOH D	20	13.244	50.277	14.738	1.00	18.37		O
	ATOM	3615	O	HOH D	21	36.790	15.994	26.963	1.00	17.85		O
	ATOM	3618	O	HOH D	22	22.367	36.375	23.529	1.00	17.11		O
	ATOM	3621	O	HOH D	23	18.911	31.260	28.272	1.00	26.39		O
20	ATOM	3624	O	HOH D	24	31.505	1.688	3.641	1.00	20.97		O
	ATOM	3627	O	HOH D	25	21.210	33.851	24.000	1.00	21.28		O
	ATOM	3630	O	HOH D	26	23.386	-0.715	22.103	1.00	15.14		O
	ATOM	3633	O	HOH D	27	29.074	-11.945	31.532	1.00	24.32		O
	ATOM	3636	O	HOH D	28	25.268	40.455	23.574	1.00	23.95		O
25	ATOM	3639	O	HOH D	29	33.156	-9.344	11.616	1.00	20.79		O
	ATOM	3642	O	HOH D	30	38.478	-5.911	7.639	1.00	21.26		O
	ATOM	3645	O	HOH D	31	24.308	53.095	18.780	1.00	19.70		O
	ATOM	3648	O	HOH D	32	17.754	48.477	15.499	1.00	20.76		O
	ATOM	3651	O	HOH D	33	42.790	5.854	23.394	1.00	23.30		O
30	ATOM	3654	O	HOH D	34	27.861	15.691	24.120	1.00	23.40		O
	ATOM	3657	O	HOH D	35	35.797	12.184	4.416	1.00	21.31		O
	ATOM	3660	O	HOH D	36	30.360	-0.992	28.013	1.00	16.72		O
	ATOM	3663	O	HOH D	37	28.276	-16.367	24.146	1.00	20.65		O
	ATOM	3666	O	HOH D	38	32.226	24.366	27.005	1.00	23.68		O
35	ATOM	3669	O	HOH D	39	20.858	29.760	23.162	1.00	21.99		O
	ATOM	3672	O	HOH D	40	49.235	18.819	15.583	1.00	25.35		O
	ATOM	3675	O	HOH D	41	24.467	-3.793	21.863	1.00	22.04		O
	ATOM	3678	O	HOH D	42	26.332	-5.940	25.774	1.00	16.62		O
	ATOM	3681	O	HOH D	43	40.578	4.994	6.108	1.00	25.58		O
40	ATOM	3684	O	HOH D	44	20.863	46.163	17.522	1.00	25.28		O
	ATOM	3687	O	HOH D	45	42.794	2.619	9.388	1.00	23.00		O
	ATOM	3690	O	HOH D	46	20.611	25.977	15.514	1.00	25.08		O
	ATOM	3693	O	HOH D	47	24.778	21.030	19.875	1.00	21.37		O
	ATOM	3696	O	HOH D	48	24.759	-5.920	23.503	1.00	16.11		O
45	ATOM	3699	O	HOH D	49	36.889	24.475	8.631	1.00	23.03		O
	ATOM	3702	O	HOH D	50	20.215	49.361	14.399	1.00	22.50		O
	ATOM	3705	O	HOH D	51	47.164	-5.215	12.615	1.00	22.87		O
	ATOM	3708	O	HOH D	52	46.004	25.078	19.448	1.00	25.71		O
	ATOM	3711	O	HOH D	53	20.097	31.594	19.238	1.00	27.29		O
50	ATOM	3714	O	HOH D	54	19.046	1.435	16.404	1.00	27.34		O
	ATOM	3717	O	HOH D	55	39.089	23.057	7.816	1.00	28.18		O
	ATOM	3720	O	HOH D	56	22.799	49.789	12.926	1.00	27.76		O
	ATOM	3723	O	HOH D	57	21.681	37.631	15.807	1.00	23.46		O
	ATOM	3726	O	HOH D	58	44.307	22.404	22.181	1.00	23.37		O
55	ATOM	3729	O	HOH D	59	46.170	-13.085	20.941	1.00	17.48		O
	ATOM	3732	O	HOH D	60	36.365	41.071	5.729	1.00	27.02		O
	ATOM	3735	O	HOH D	61	36.990	7.023	22.512	1.00	22.71		O
	ATOM	3738	O	HOH D	62	19.169	-8.584	16.372	1.00	23.92		O
	ATOM	3741	O	HOH D	63	20.055	28.469	14.698	1.00	26.12		O
60	ATOM	3744	O	HOH D	64	30.998	-12.907	29.623	1.00	25.28		O
	ATOM	3747	O	HOH D	65	37.347	-1.150	7.121	1.00	22.93		O
	ATOM	3750	O	HOH D	66	33.031	-13.258	27.775	1.00	32.50		O
	ATOM	3753	O	HOH D	67	22.945	-4.003	11.274	1.00	30.88		O

	ATOM	3756	O	HOH D	68	27.701	41.270	1.720	1.00	29.57		O
	ATOM	3759	O	HOH D	69	25.980	-9.896	14.868	1.00	27.31		O
	ATOM	3762	O	HOH D	70	23.821	12.814	14.658	1.00	24.54		O
	ATOM	3765	O	HOH D	71	35.006	43.534	25.083	1.00	28.85		O
5	ATOM	3768	O	HOH D	72	35.312	36.253	1.522	1.00	29.21		O
	ATOM	3771	O	HOH D	73	48.598	-9.901	24.935	1.00	25.55		O
	ATOM	3774	O	HOH D	74	42.294	-13.685	16.218	1.00	25.18		O
	ATOM	3777	O	HOH D	75	42.607	12.387	16.515	1.00	31.10		O
	ATOM	3780	O	HOH D	76	26.330	35.006	4.050	1.00	31.74		O
10	ATOM	3783	O	HOH D	77	32.850	10.209	3.504	1.00	27.19		O
	ATOM	3786	O	HOH D	78	30.508	10.747	29.512	1.00	25.87		O
	ATOM	3789	O	HOH D	79	45.693	19.098	22.237	1.00	30.36		O
	ATOM	3792	O	HOH D	80	15.634	44.761	11.710	1.00	26.02		O
	ATOM	3795	O	HOH D	81	18.085	50.959	3.872	1.00	35.51		O
15	ATOM	3798	O	HOH D	82	29.549	1.503	7.572	1.00	29.19		O
	ATOM	3801	O	HOH D	83	39.725	31.841	30.695	1.00	40.77		O
	ATOM	3804	O	HOH D	84	20.283	36.188	-4.205	1.00	39.38		O
	ATOM	3807	O	HOH D	85	34.763	-11.883	13.146	1.00	21.47		O
	ATOM	3810	O	HOH D	86	26.410	32.901	7.289	1.00	24.64		O
20	ATOM	3813	O	HOH D	87	44.314	-2.758	11.932	1.00	23.95		O
	ATOM	3816	O	HOH D	88	30.034	-14.313	17.413	1.00	29.20		O
	ATOM	3819	O	HOH D	89	26.961	12.263	27.391	1.00	30.17		O
	ATOM	3822	O	HOH D	90	28.249	0.678	3.312	1.00	28.11		O
	ATOM	3825	O	HOH D	91	45.718	32.030	14.220	1.00	34.46		O
25	ATOM	3828	O	HOH D	92	28.299	-9.696	27.995	1.00	24.79		O
	ATOM	3831	O	HOH D	93	13.832	48.982	7.768	1.00	33.46		O
	ATOM	3834	O	HOH D	94	43.000	-11.174	31.241	1.00	28.43		O
	ATOM	3837	O	HOH D	95	35.944	8.335	1.385	1.00	29.40		O
	ATOM	3840	O	HOH D	96	29.165	29.895	11.877	1.00	24.28		O
30	ATOM	3843	O	HOH D	97	32.349	31.864	24.473	1.00	30.09		O
	ATOM	3846	O	HOH D	98	22.954	24.601	11.686	1.00	28.72		O
	ATOM	3849	O	HOH D	99	31.154	51.462	19.574	1.00	25.81		O
	ATOM	3852	O	HOH D	100	43.443	12.360	23.615	1.00	24.55		O
	ATOM	3855	O	HOH D	101	15.670	52.252	4.362	1.00	34.13		O
35	ATOM	3858	O	HOH D	102	25.701	41.081	26.231	1.00	27.56		O
	ATOM	3861	O	HOH D	103	37.527	21.694	22.195	1.00	32.29		O
	ATOM	3864	O	HOH D	104	33.325	-12.660	37.738	1.00	35.18		O
	ATOM	3867	O	HOH D	105	26.319	5.217	15.262	1.00	26.13		O
	ATOM	3870	O	HOH D	106	33.848	22.140	26.173	1.00	31.07		O
40	ATOM	3873	O	HOH D	107	35.489	18.857	24.618	1.00	27.43		O
	ATOM	3876	O	HOH D	108	42.855	46.462	8.947	1.00	33.41		O
	ATOM	3879	O	HOH D	109	42.188	5.317	9.853	1.00	30.90		O
	ATOM	3882	O	HOH D	110	41.401	45.084	19.630	1.00	35.22		O
	ATOM	3885	O	HOH D	111	45.990	-4.685	27.447	1.00	36.33		O
45	ATOM	3888	O	HOH D	112	44.969	4.979	13.641	1.00	30.89		O
	ATOM	3891	O	HOH D	113	21.231	24.488	19.771	1.00	29.91		O
	ATOM	3894	O	HOH D	114	28.991	22.460	25.768	1.00	32.28		O
	ATOM	3897	O	HOH D	115	30.182	42.704	28.664	1.00	34.73		O
	ATOM	3900	O	HOH D	116	38.457	26.788	9.301	1.00	28.25		O
50	ATOM	3903	O	HOH D	117	33.010	8.247	32.080	1.00	30.38		O
	ATOM	3906	O	HOH D	118	40.296	-12.388	19.763	1.00	29.43		O
	ATOM	3909	O	HOH D	119	26.522	44.371	25.621	1.00	29.51		O
	ATOM	3912	O	HOH D	120	43.804	-4.826	10.570	1.00	33.46		O
	ATOM	3915	O	HOH D	121	47.448	-11.680	26.748	1.00	37.40		O
55	ATOM	3918	O	HOH D	122	40.716	-13.572	24.920	1.00	24.40		O
	ATOM	3921	O	HOH D	123	41.998	-1.274	34.849	1.00	32.74		O
	ATOM	3924	O	HOH D	124	45.154	42.318	18.028	1.00	36.95		O
	ATOM	3927	O	HOH D	125	30.324	-11.134	10.862	1.00	29.46		O
	ATOM	3930	O	HOH D	126	42.517	10.179	15.159	1.00	30.78		O
60	ATOM	3933	O	HOH D	127	48.214	-11.222	16.932	1.00	31.45		O
	ATOM	3936	O	HOH D	128	23.815	-9.373	14.042	1.00	33.96		O
	ATOM	3939	O	HOH D	129	31.988	24.965	29.884	1.00	32.47		O
	ATOM	3942	O	HOH D	130	35.266	30.662	4.339	1.00	37.13		O

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ATOM   3945  O   HOH D 131      42.057  38.530  10.976  1.00 38.75           O
ATOM   3948  O   HOH D 132      24.900   3.888  13.671  1.00 41.30           O
ATOM   3951  O   HOH D 133      44.797 -11.819  18.372  1.00 31.27           O
ATOM   3954  O   HOH D 134      31.380  27.561   6.462  1.00 38.93           O
ATOM   3957  O   HOH D 135      24.585  -2.131   6.886  1.00 36.52           O
ATOM   3960  O   HOH D 136      44.178  14.598  21.666  1.00 49.82           O
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CLAIMS

1. An Fve polypeptide comprising at least one biological activity of native Fve protein, and being a fragment, homologue, variant or derivative thereof.

2. An Fve polypeptide according to Claim 1, which comprises an immunomodulatory activity.

3. An Fve polypeptide according to Claim 1 or 2, which comprises a biological activity selected from the group consisting of: up-regulation of expression of Th1/Tc1 cytokines, preferably IFN-γ and TNF-α, down-regulation of expression of Th2/Tc2 cytokines, preferably IL-4 and IL-13, up-regulation of expression of T regulatory (Tr) cytokines IL-10 and TGF-β, hemagglutination activity, cell aggregation activity, lymphocyte aggregation activity, lymphoproliferation activity, up-regulation of expression of IL-2, IFN-γ, TNF-α, but not IL-4 in $CD3^+$ T cells, interaction with T and NK cells, adjuvant activity, stimulation of $CD3^+$ $CD16^+$ $CD56^+$ natural killer (NK) T cells and $CD3^+$ $CD8^+$ $CD18^{+\,bright}$ T cells, and up-regulation of allergen specific Th1 immune responses.

3. An Fve polypeptide according to Claim 1, 2 or 3, in which the polypeptide comprises between 2 to 20 residues of amino acid sequence flanking the glycine residue corresponding to position 28 of Fve.

4. An Fve polypeptide according to any preceding claim, in which the polypeptide comprises the sequence RGT or the sequence RGD.

5. An Fve polypeptide according to any preceding claim, in which the polypeptide has a sequence as set out in **Appendix A** or **Appendix B**.

6. An Fve polypeptide comprising an sequence selected from the group consisting of: Fve R27A, Fve T29A, GST-Fve (wild type), GST-Fve R27A, and GST-Fve T29A, and fragments, homologues, variants and derivatives thereof.

7. A polypeptide comprising a first portion comprising at least a fragment of native Fve, or an Fve polypeptide according to any preceding claim, and a second portion comprising at least a fragment of an allergen.

8. A polypeptide according to Claim 7, in which the allergen comprises an allergen from a mite, preferably from Family *Glycyphagidae* or Family *Pyroglyphidae*, preferably a group 1 allergen (Der p 1, Der f 1, Blo t 1, Eur m1, Lep d 1), a group 2 allergen (Der p 2, Der f 2, Blo t 2, Eur m 2, Lep d 2), a group 5 allergen (Blo t 5, Der p 5, Der f 5, Eur m 5, Lep d 5) a group 15 allergen (Der p 15, Der f 15, Blot 15, Eur m 15, Lep d 15).

9. A Fve polypeptide or a polypeptide according to Claim 7 or 8, which is selected from the group consisting of: Blo t 5-Fve, Blo t 5-FveR27A, Blo t 5-FveT29A, Der p 2-FveT29A, GST-Der p 2-FveR27A, GST-Der p 2-FveT29A, Blo t 5-Der p 2-FveR27A and Blo t 5-Der p 2-FveT29A.

10. A polypeptide according to Claim 7, in which the allergen is selected from the group consisting of: tree pollen allergen, Bet v 1 and Bet v 2 from birch tree; grass pollen allergen, Phl p 1 and Phl p 2 from timothy grass; weed pollen allergen, antigen E from ragweed; major feline antigen, Fel d; major fungal allergen, Asp f1, Asp f2, and Asp f3 from *Aspergillus fumigatus*.

11. A polypeptide comprising a first portion comprising at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second portion comprising at least a fragment of a viral antigen selected from the group consisting of: E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; and Tax from HTLV-1.

12. A polypeptide according to Claim 11, which comprises HCV Core23-FveT29A, or HPV E7-FveT29A.

13. A polypeptide comprising a first portion comprising at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second portion comprising at least a fragment of a tumour-associated antigen selected from the group consisting of: MAGE-1, MAGE-2, MAGE-3, preferably a sequence, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, and P15.

14. A polypeptide according to Claim 13, which comprises MAGE3-FveT29A, MART1-FveT29A or CEA-FveT29A.

15. A nucleic acid encoding a Fve polypeptide or a polypeptide according to any preceding claim.

16. A nucleic acid according to Claim 15, in which the nucleic acid comprises CGT GGT ACC, or a sequence which differs from the above by virtue of the degeneracy of the genetic code and which encodes a sequence RGT.

17. A nucleic acid comprising a first sequence encoding at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second sequence encoding at least a fragment of an allergen.

18. A nucleic acid according to Claim 17, which comprises Blo t 5-Fve, Blo t 5-FveR27A, Blo t 5-FveT29A, Der p 2-FveT29A, GST-Der p 2-FveR27A, GST-Der p 2-FveT29A, Blo t 5-Der p 2-FveR27A or Blo t 5-Der p 2-FveT29A.

19. A nucleic acid comprising a first sequence encoding at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second sequence encoding at least a fragment of a viral antigen selected from the group consisting of: E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; and Tax from HTLV-1.

20. A nucleic acid according to Claim 19, which comprises HCV Core23-FveT29A, or HPV E7-FveT29A.

21. A nucleic acid comprising a first sequence encoding at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second sequence encoding at least a fragment of a tumour associated antigen selected from the group consisting of: MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, and P15.

22. A nucleic acid according to Claim 21, which comprises MAGE3-FveT29A, MART1-FveT29A or CEA-FveT29A.

23. A nucleic acid selected from the group consisting of: Fve R27A, Fve T29A, GST-Fve (wild type), GST-Fve R27A, GST-Fve T29A, Blo t 5-Fve, Blo t 5-FveR27A, Blo t 5-FveT29A, GST-Der p 2-FveR27A, GST-Der p 2-FveT29A, Blo t 5-Der p 2-FveR27A, Blo t 5-Der p 2-FveT29A, and fragments, homologues, variants and derivatives thereof.

24. A vector, preferably an expression vector, comprising a nucleic acid sequence according to any of Claims 15 to 23.

25. A DNA vaccine comprising a nucleic acid encoding Fve, a nucleic acid according to any of Claims 15 to 23, or a vector according to Claim 24.

26. A host cell comprising a nucleic acid encoding Fve, a nucleic acid according to any of Claims 15 to 23, or a vector according to Claim 24.

27. A transgenic non-human organism comprising a nucleic acid encoding Fve, a nucleic acid according to any of Claims 15 to 23, or a vector according to Claim 24.

28. A transgenic non-human organism according to Claim 27 which is a bacterium, a yeast, a fungus, a plant or an animal, preferably a mouse.

29. A pharmaceutical composition comprising a polypeptide according to any of Claims 1 to 14, a nucleic acid according to any of Claims 15 to 23, a vector according to Claim 24, a DNA vaccine according to Claim 25, or a host cell according to Claim 26, together with a pharmaceutically acceptable carrier or diluent.

30. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 as an immumodulator.

31. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 to enhance an immune response in a mammal.

32. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 to stimulate proliferation of $CD3^+$ $CD8^+$ $CD18^{+\,bright}$ T cells.

33. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 to stimulate proliferation of $CD3^+$ $CD16^+$ $CD56^+$ natural killer (NK) T cells.

34. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 to stimulate production of IL-2, IL-10, TGF-β, IFN-γ or TNF-α in $CD3^+$ cells.

35. Use according to Claim 34, in which production of IL-4 is not stimulated in the $CD3^+$ cells.

36. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 as an adjuvant for a vaccine.

37. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29 in a method of treatment or prophylaxis of a disease.

38. Use of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector or host cell according to any of Claims 1 to 29 for the preparation of a pharmaceutical composition for the treatment of a disease.

39. A method of treating an individual suffering from a disease or preventing the occurrence of a disease in an individual, the method comprising administering to the individual a therapeutically or prophylactically effective amount of a native Fve polypeptide, or an Fve polypeptide, nucleic acid, vector, DNA vaccine, host cell, transgenic organism, or a pharmaceutical composition according to any of Claims 1 to 29.

40. A use or method according to any of Claims 37, 38 or 39, in which the disease comprises an atopic disease or allergy.

41. Use of a DNA vaccine according to Claim 25, preferably as dependent on Claim 17 or 18, in a method of treatment or prevention of an allergy.

42. A use or method according to Claim 40 or 41, in which the allergy is selected from the group consisting of: allergic asthma, a seasonal respiratory allergy, a perennial respiratory allergy, allergic rhinitis, hayfever, nonallergic rhinitis, vasomotor rhinitis, irritant rhinitis, an allergy against grass pollen, weed pollen, tree pollen or animal danders, an allergy associated with allergic asthma and a food allergy.

43. A use or method according to Claim 40, 41 or 42, in which the allergy is to a house dust mite from Family Glyphagidae, preferably *Blomia tropicalis* or from Family Pyroglyphidae, preferably *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*, or to fungi or fungal spores, preferably *Aspergillus fumigatus*, or to tree pollen allergens, preferably from birch tree, or grass pollen allergens, preferably from timothy grass, or weed allergens, preferably ragweed.

44. A use or method according to any of Claims 37, 38 or 39, in which the disease comprises a cancer.

45. Use of a DNA vaccine according to Claim 25, preferably as dependent on Claim 19 or 20, in a method of treatment or prevention of a cancer, or in a method of suppressing tumour progression.

46. Use of a DNA vaccine according to Claim 25, preferably as dependent on Claim 21, in a method of treatment or prevention of a cancer, or in a method of suppressing tumour progression.

47. A use or method according to Claim 44, 45 or 46, in which the cancer comprises a T cell lymphoma, melanoma, lung cancer, colon cancer, breast cancer or prostate cancer.

48. A method of identifying a molecule capable of binding to Fve, the method comprising exposing a native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism according to any of Claims 1 to 24, 26 and 27 to a candidate molecule and detecting whether the candidate molecule binds to the native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism.

49. A method of identifying an agonist or antagonist of an Fve polypeptide, the method comprising: (a) providing a cell or organism; (b) exposing the cell or organism to a native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism according to any of Claims 1 to 24, 26 and 27; (c) exposing the cell to a candidate molecule; and (d) detecting an Fve mediated effect.

50. A method according to Claim 49, in which the Fve mediated effect is selected from the biological activities set out in Claim 2.

51. A method according to Claim 48, 49 or 50, in which the method further comprises isolating or synthesising a selected or identified molecule.

52. A molecule identified or selected using a method according to any of Claims 48 to 51.

53. A native Fve polypeptide, or an Fve polypeptide in crystalline form.

54. A native Fve polypeptide, or an Fve polypeptide in crystalline form according to Claim 53, which has the structural coordinates shown in **Appendix C**.

55. A model for at least part of Fve made using a crystal according to Claim 53 or 54.

56. A method of screening for a receptor capable of binding to Fve, or designing a ligand capable of modulating the interaction between Fve and an Fve receptor, comprising the use of a model according to Claim 55.

57. A computer readable medium having stored thereon the structure of a crystal according to Claim 53 or 54, or a model according to Claim 55.

58. A ligand identified by the method according to Claim 56.

59. Use of a molecule according to Claim 52 or a ligand according to Claim 58 for the treatment or prevention of a disease in an individual.

60. A pharmaceutical composition comprising a molecule according to Claim 52 or a ligand according to Claim 58 and optionally a pharmaceutically acceptable carrier, diluent, excipient or adjuvant or any combination thereof.

61. A method of treating and/or preventing a disease comprising administering a molecule according to Claim 52 or a ligand according to Claim 58 and/or a pharmaceutical composition according to Claim 60 to an individual in need of such treatment.

62. A method of amplifying a sub-population of cells, the method comprising: (a) obtaining a population of cells from an individual; (b) amplifying $CD3^+$ $CD8^+$ and $CD18^{+\ bright}$ T cells by exposing the population of cells to a native Fve polypeptide, or an Fve polypeptide or nucleic acid, vector, host cell or transgenic organism according to any of Claims 1 to 24, 26 and 27.

63. A method according to Claim 62, further comprising the step of: (c) isolating the $CD3^+$ $CD8^+$ and $CD18^{+\ bright}$ T cells.

64. A method of treating an individual suffering from a disease or preventing the occurrence of a disease in an individual, the method comprising amplifying a $CD3^+$ $CD8^+$ and $CD18^{+\,bright}$ T cell by a method according to Claim 62 or 63, and administering the amplified $CD3^+$ $CD8^+$ and $CD18^{+\,bright}$ T cell to an individual.

65. An amplified population of $CD3^+$ $CD8^+$ and $CD18^{+\,bright}$ T cells obtainable by a method according to Claim 62 or 63.

66. A pharmaceutical composition comprising an amplified population of $CD3^+$ $CD8^+$ and $CD18^{+\,bright}$ T cells according to Claim 65, together with a pharmaceutically acceptable excipient or carrier.

67. A combination comprising a first component comprising an immunomodulator and a second component comprising at least a fragment of an allergen, a viral antigen or a tumour associated antigen.

68. A combination according to Claim 67 in which the first component is separate from the second component.

69. A combination according to Claim 67 in which the first component is associated with the second component.

70. A combination according to Claim 67 which is a fusion protein.

71. A combination according to Claim 67, in which the first component comprises a native Fve polypeptide, or a polypeptide according to any of Claims 1 to 14.

72. A combination according to any of Claims 67 to 71, in which the second component comprises an allergen selected from the group consisting of: a mite allergen, an mite allergen from Family *Glycyphagidae* or Family *Pyroglyphidae*, a group 1 allergen

(Der p 1, Der f 1, Blo t 1, Eur m1, Lep d 1), a group 2 allergen (Der p 2, Der f 2, Blo t 2, Eur m 2, Lep d 2), a group 5 allergen (Blo t 5, Der p 5, Der f 5, Eur m 5, Lep d 5), a group 15 allergen (Der p 15, Der f 15, Blot 15, Eur m 15, Lep d 15), a tree pollen allergen, Bet v 1 and Bet v 2 from birch tree; grass pollen allergen, Phl p 1 and Phl p 2 from timothy grass; weed pollen allergen, antigen E from ragweed; major feline antigen, Fel d; major fungal allergen, Asp f1, Asp f2, and Asp f3 from *Aspergillus fumigatus*.

73. A combination according to any of Claims 67 to 71, in which the second component comprises a viral antigen selected from the group consisting of: E6 and E7 from HPV; core Ag and E2 from HCV; core and surface antigens from HBV; LMP-1, LMP-2, EBNA-2, EBNA-3 from EBV; and Tax from HTLV-1.

74. A combination according to any of Claims 67 to 71, in which the second component comprises a tumour-associated antigen selected from the group consisting of: MAGE-1, MAGE-2, MAGE-3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proteinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, BTA, GnT-V, β-catenin, CDK4, and P15.

75. An immunomodulator-antigen conjugate, preferably an immunomodulator-allergen conjugate, an immunomodulator-tumour associated antigen conjugate or a immunomodulator-viral antigen conjugate, in which the immunomodulator preferably comprises an Fve polypeptide.

76. A polypeptide comprising a first portion comprising at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second portion comprising at least a fragment of a viral antigen selected from the group consisting of an antigen from from Adenovirus, Parainfluenza 3 virus, Human Immunodeficiency Virus (HIV), Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, or Influenza A, Flu A.

77. A nucleic acid comprising a first sequence encoding at least a fragment of native Fve, or an Fve polypeptide according to any of Claims 1 to 6, and a second sequence encoding at least a fragment of a viral antigen selected from the group consisting of an antigen from from Adenovirus, Parainfluenza 3 virus, Human Immunodeficiency Virus (HIV), Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, or Influenza A, Flu A.

78. A combination according to any of Claims 67 to 71, in which the second component comprises a tumour-associated antigen selected from the group consisting of antigen from from Adenovirus, Parainfluenza 3 virus, Human Immunodeficiency Virus (HIV), Herpes simplex virus, HSV, Respiratory syncytial virus, RSV, or Influenza A, Flu A.

79. A nucleic acid sequence, including an Fve nucleic acid sequence, a polypeptide sequence, including a Fve polypeptide sequence, a method of treatment, a method of diagnosis, a host cell, vector, transgenic animal, a transgenic plant, a genetically-modified lactose bacilli, assay, vaccine, phamaceutical composition or agent substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

ABSTRACT

MOLECULES

We describe an Fve polypeptide being a fragment, homologue, variant or derivative of Fve protein, which comprises at least one biological activity of Fve protein. uses of such a polypeptide, etc, and nucleic acids encoding these, in the treatment and prevention of allergy and cancer are also disclosed.

Figure 1

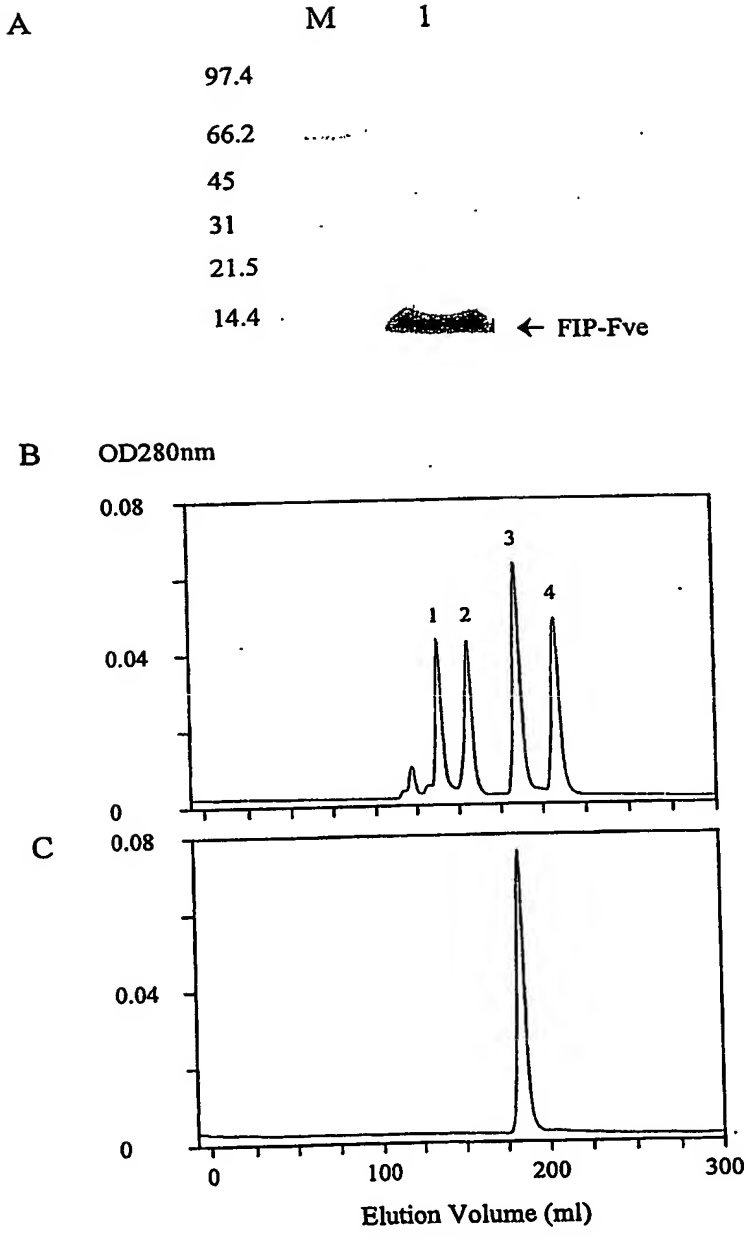

A
M
1
97.4
66.2
45
31
21.5
14.4
← FIP-Fve
B
OD280nm
0.08
0.04
0
1
2
3
4
C
0.08
0.04
0
0
100
200
300
Elution Volume (ml)

FIGURE 1

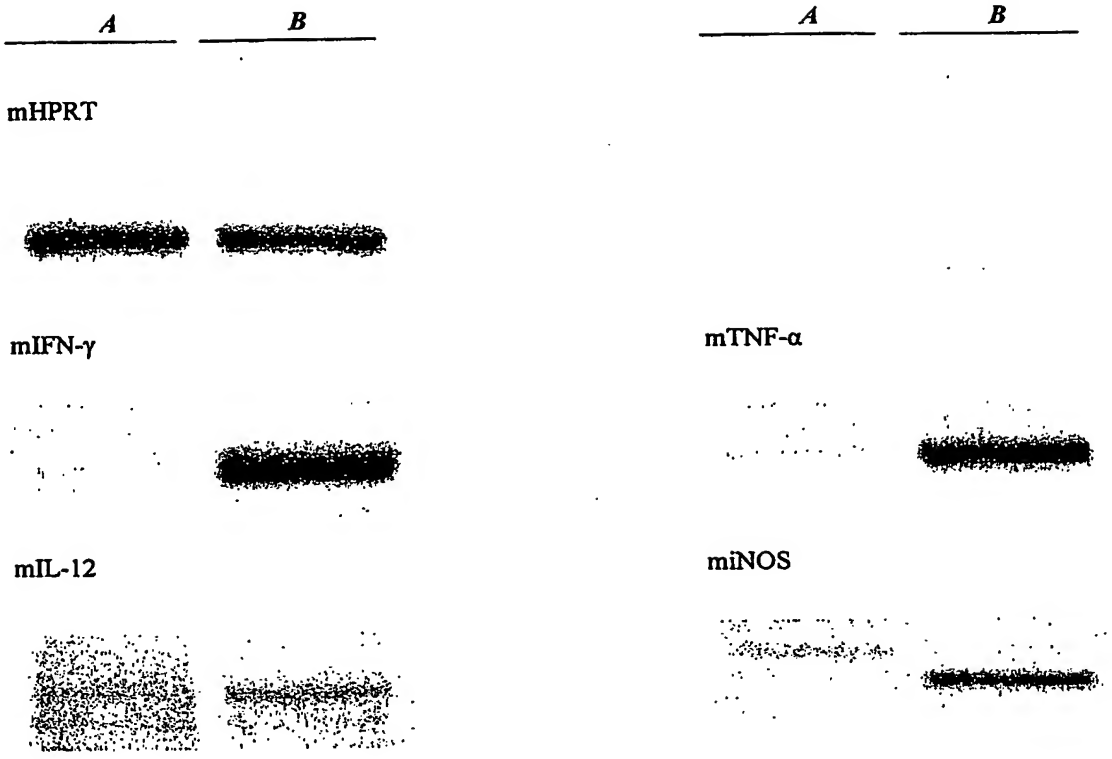


FIGURE 2

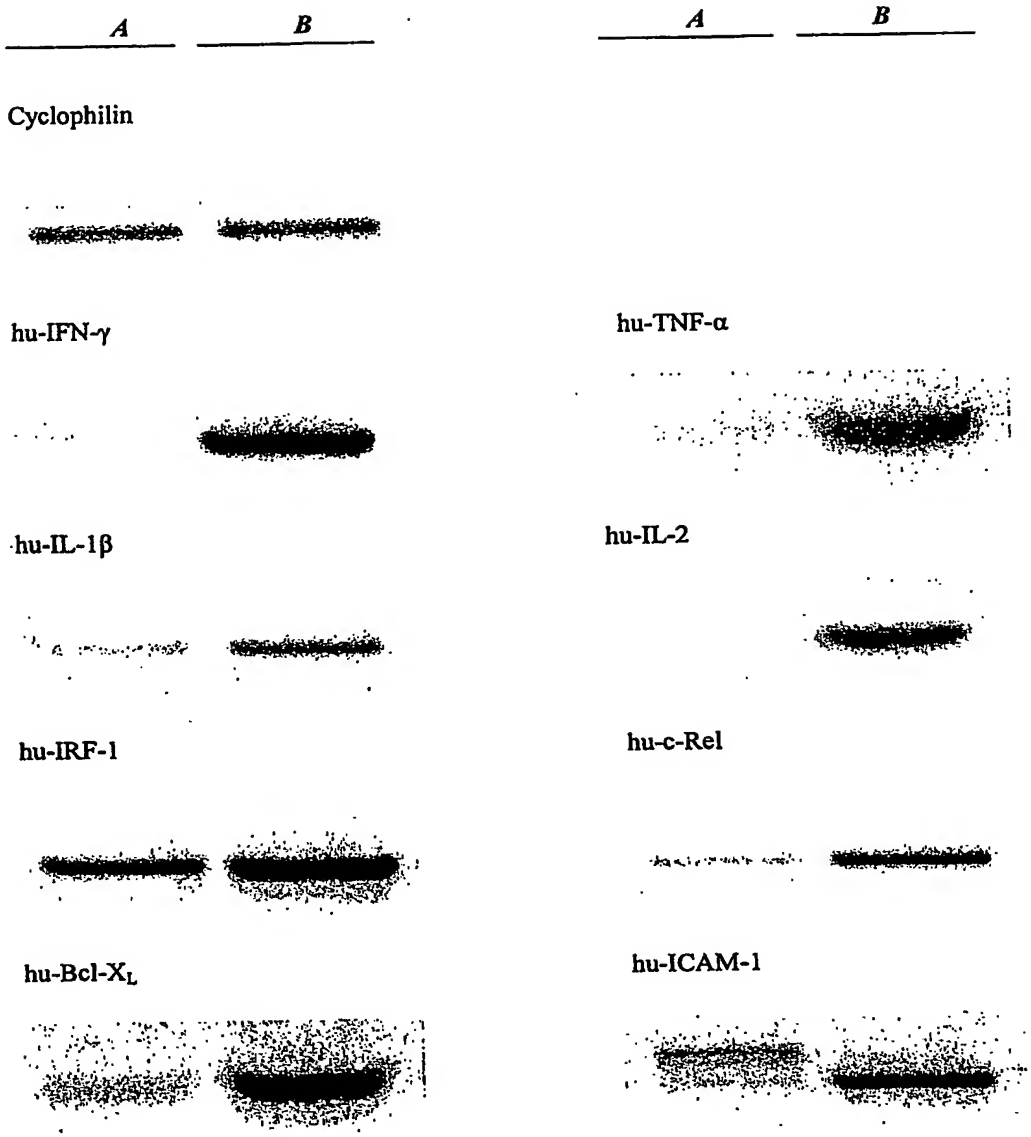
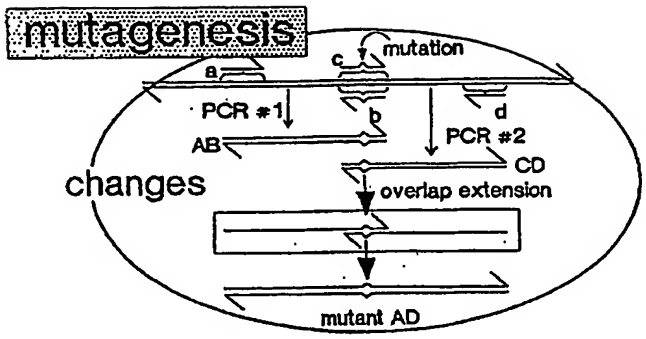

A
B
A
B
Cyclophilin
hu-IFN-γ
hu-TNF-α
hu-IL-1β
hu-IL-2
hu-IRF-1
hu-c-Rel
hu-Bcl-X$_L$
hu-ICAM-1

FIGURE 3

(A)


mutagenesis
mutation
a
c
PCR #1
b
d
PCR #2
AB
CD
changes
overlap extension
mutant AD

(B)

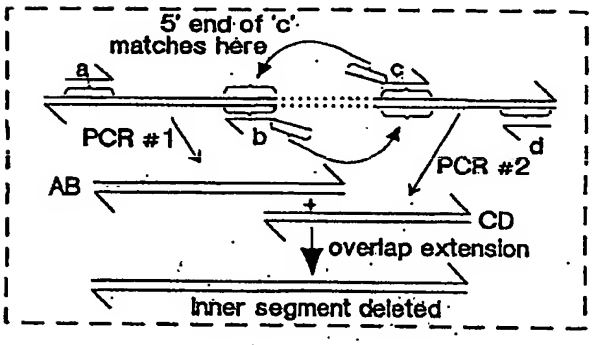

5' end of 'c'
matches here
a
c
PCR #1
b
d
PCR #2
AB
CD
overlap extension
Inner segment deleted

FIGURE 4

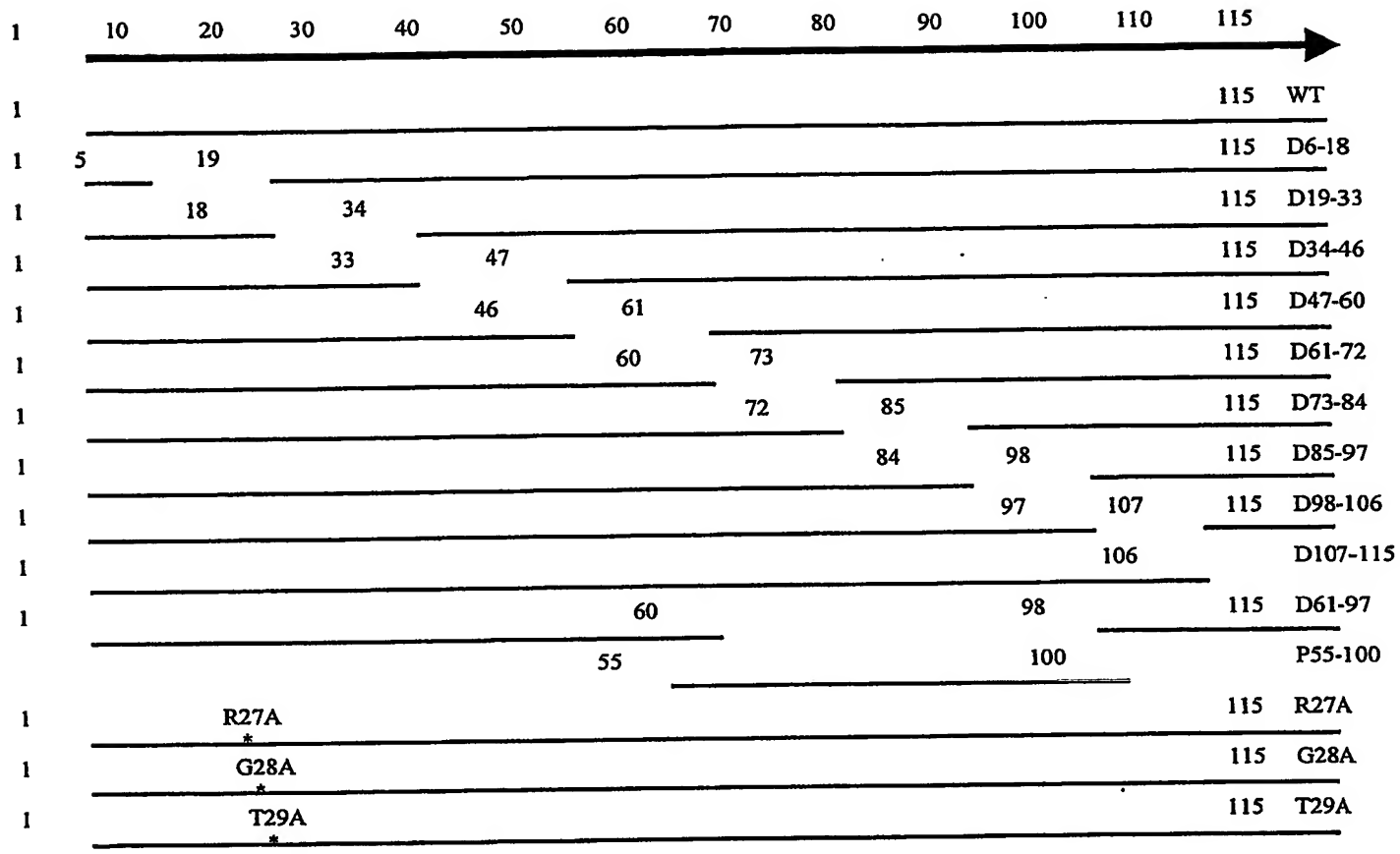

1
10
20
30
40
50
60
70
80
90
100
110
115
1 115 WT
1 5 19 115 D6-18
1 18 34 115 D19-33
1 33 47 115 D34-46
1 46 61 115 D47-60
1 60 73 115 D61-72
1 72 85 115 D73-84
1 84 98 115 D85-97
1 97 107 115 D98-106
1 106 D107-115
1 60 98 115 D61-97
55 100 P55-100
1 R27A 115 R27A
1 G28A 115 G28A
1 T29A 115 T29A

FIGURE 5

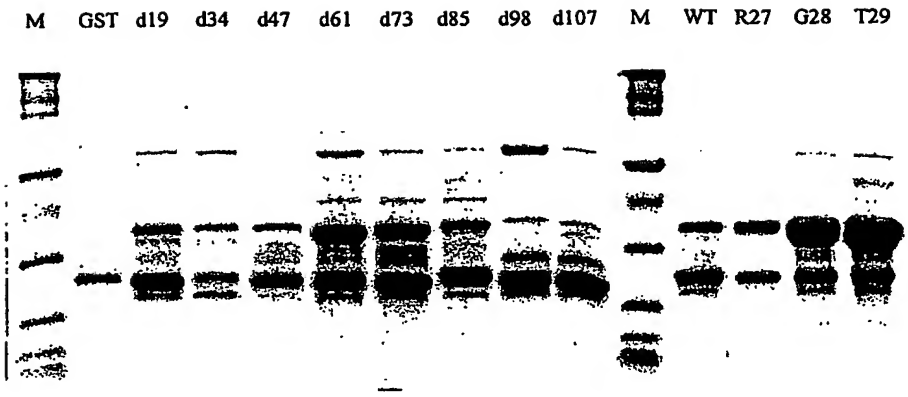


FIGURE 6

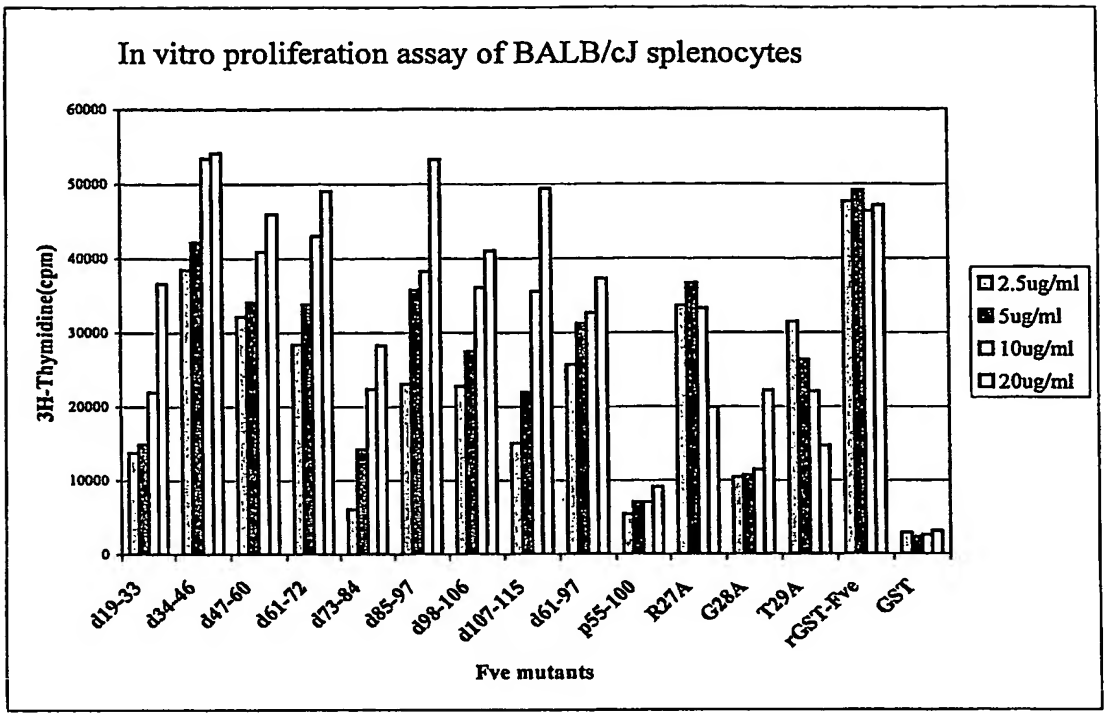

In vitro proliferation assay of BALB/cJ splenocytes
3H-Thymidine(cpm)
60000
50000
40000
30000
20000
10000
0
d19-33
d34-46
d47-60
d61-72
d73-84
d85-97
d98-106
d107-115
d61-97
p55-100
R27A
G28A
T29A
rGST-Fve
GST
Fve mutants
2.5ug/ml
5ug/ml
10ug/ml
20ug/ml

FIGURE 7

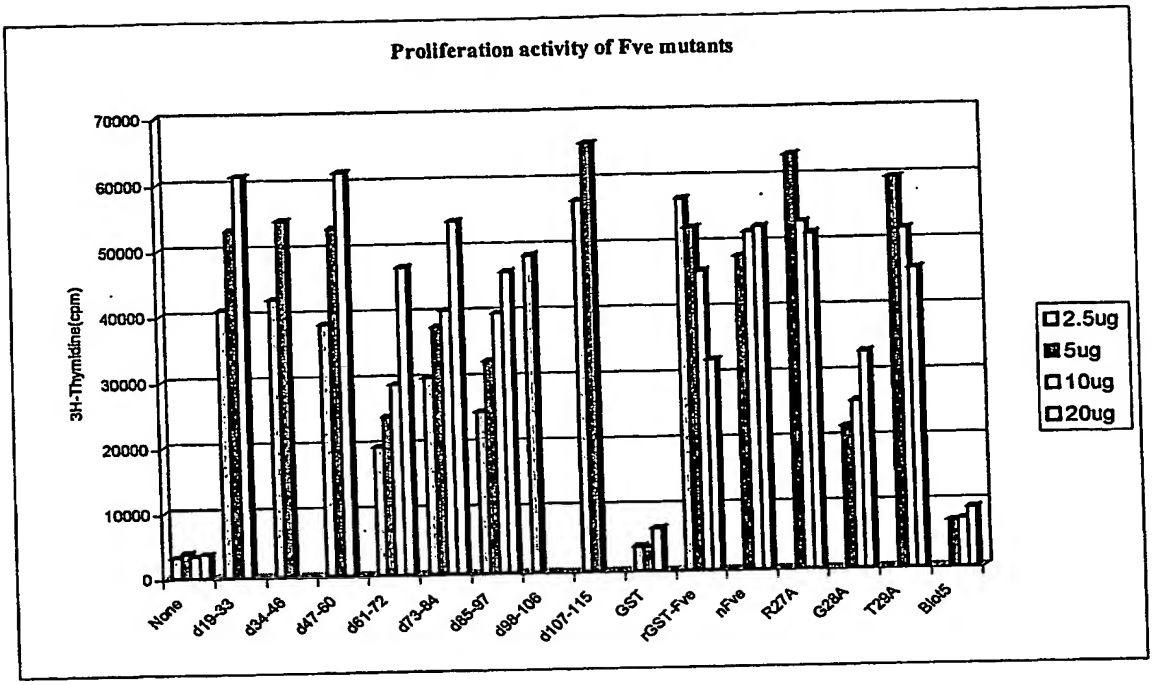
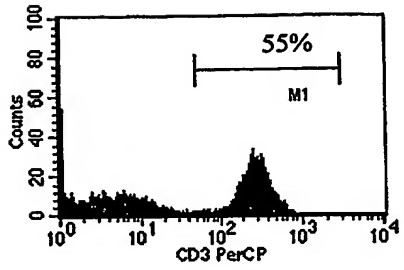
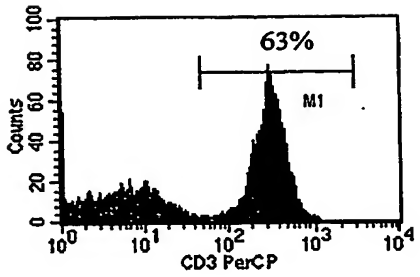

Proliferation activity of Fve mutants
3H-Thymidine(cpm)
70000
60000
50000
40000
30000
20000
10000
0
None
d19-33
d34-46
d47-60
d61-72
d73-84
d85-97
d98-106
d107-115
GST
rGST-Fve
nFve
R27A
G28A
T29A
Blo15
2.5ug
5ug
10ug
20ug

FIGURE 8

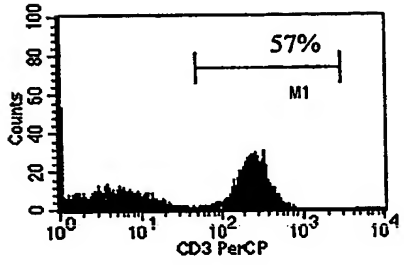
(A).



(C).



(B).



(D).

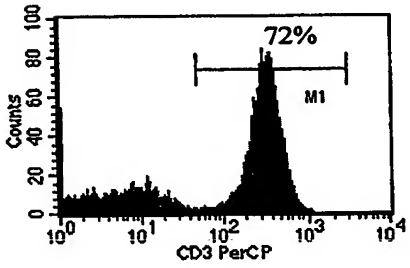
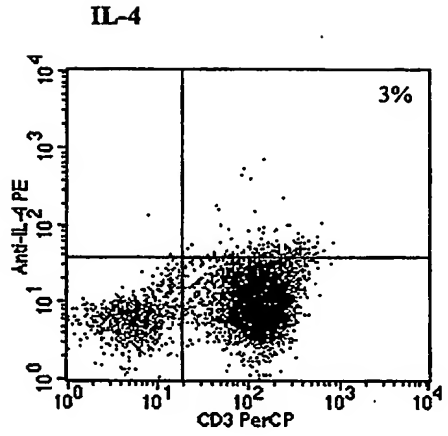
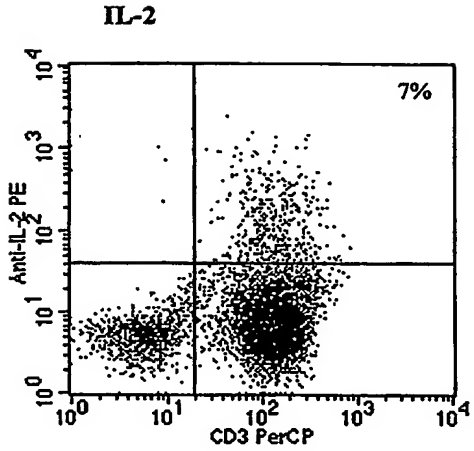


FIGURE 9

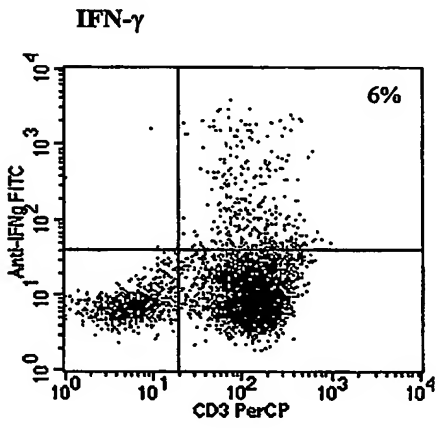
(A).



(B).



(C).



(D).

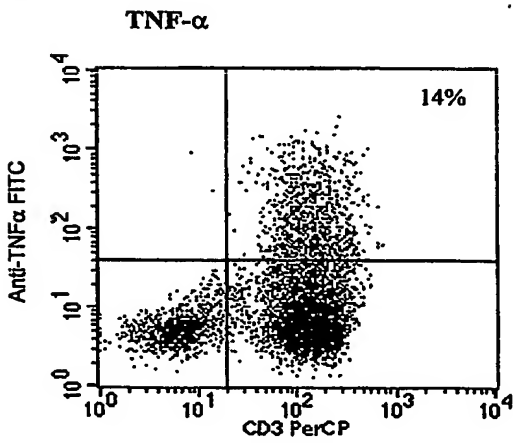
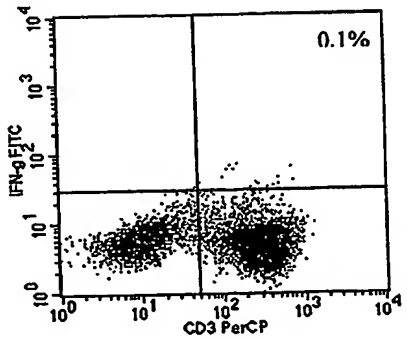


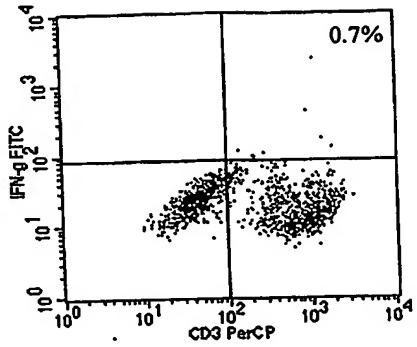
FIGURE 10

IFN-γ production at day 3

(1a).



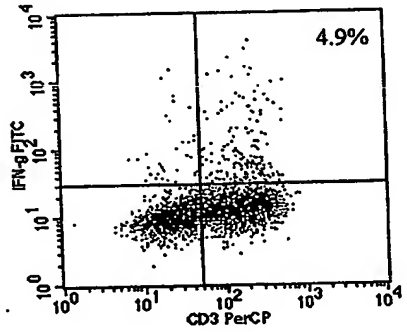
(1b).



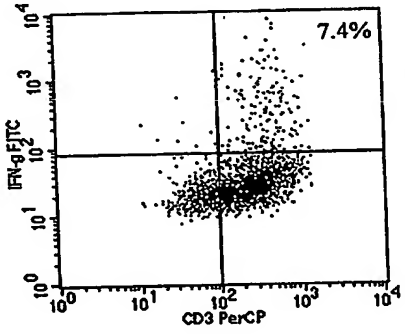
GST

0.8%

(2a).



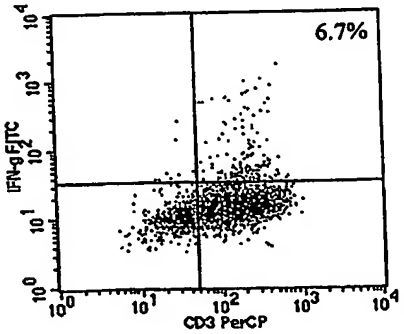
(2b).



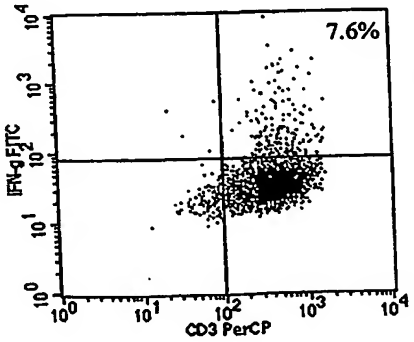
GST-FveWT

12.3%

(3a).



(3b).

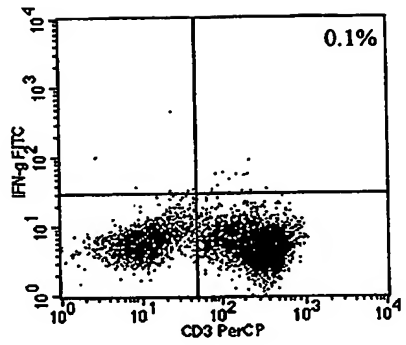


GST-FveR27A

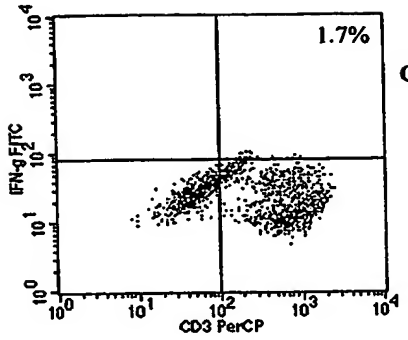
14.3%

FIGURE 11

(4a).



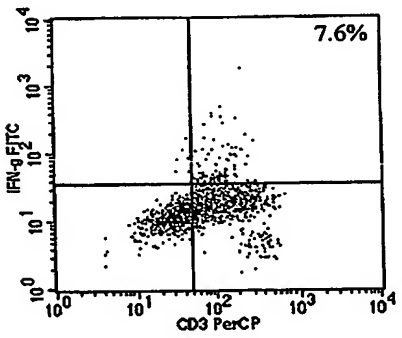
(4b).



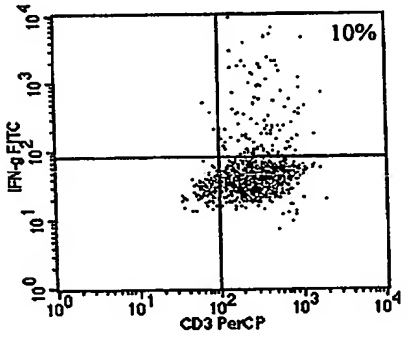
GST-FveG28A

1.8%

(5a).



(5b).



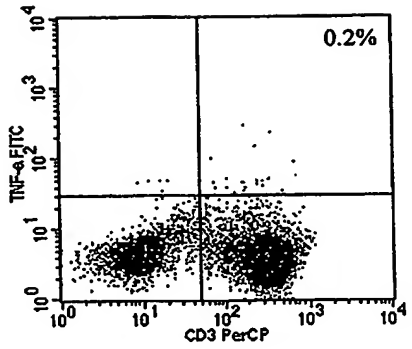
GST-FveT29A

17.6%

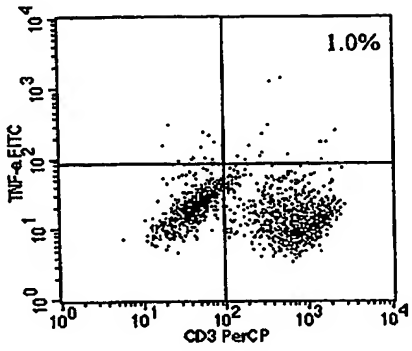
FIGURE 11 (CONTINUED)

TNF-α production at day 3

(1a).



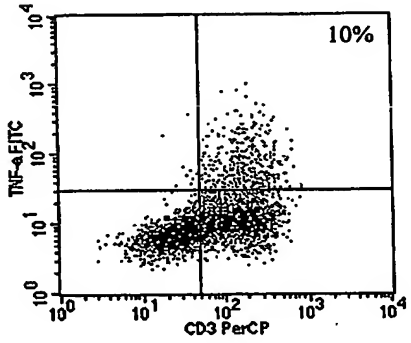
(1b).



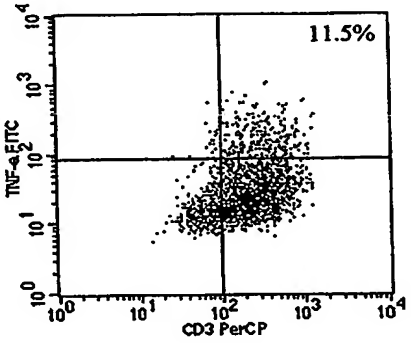
GST

1.2%

(2a).



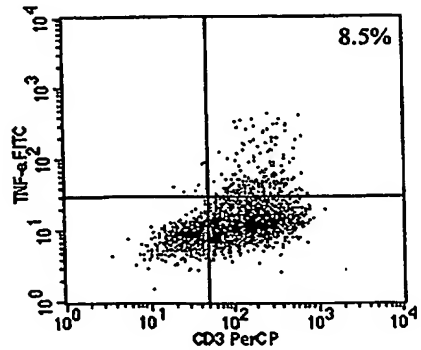
(2b).



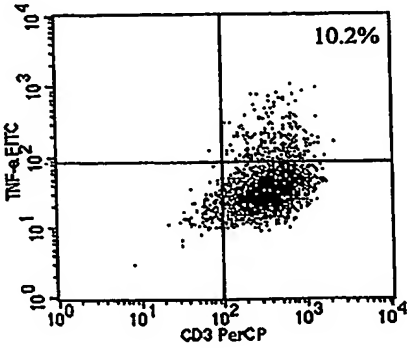
GST-FveWT

21.5%

(3a).



(3b).

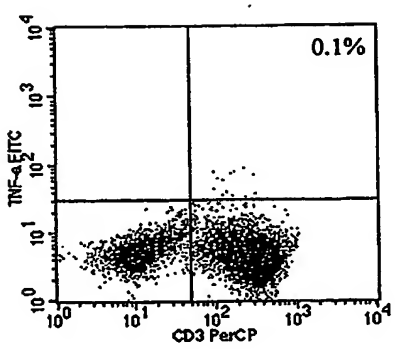


GST-FveR27A

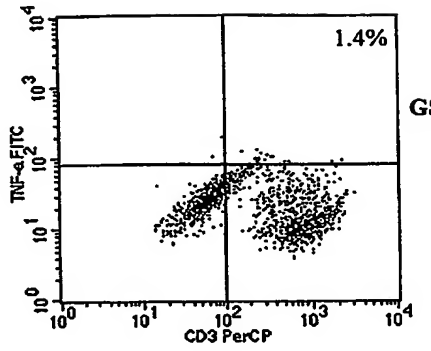
18.7%

FIGURE 12

(4a).



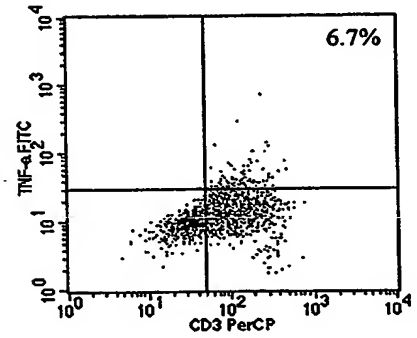
(4b).



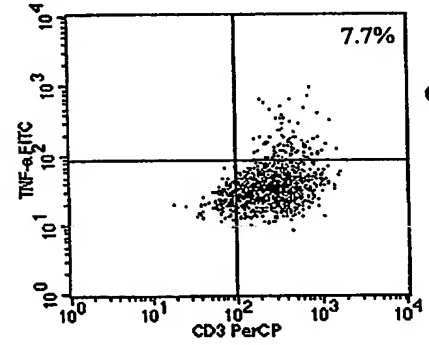
GST-FveG28A

1.5%

(5a).



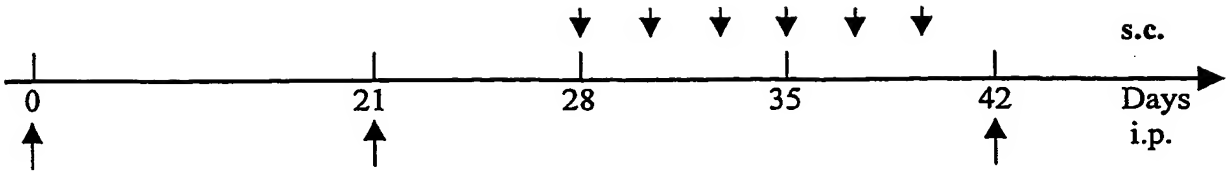
(5b).



GST-FveT29A

14.4%

FIGURE 12 (CONTINUED)



(group A)

ip(10ug of Derp2+Alum) ip(10ug of Derp2+Alum) sc(50ug of Derp2 alone) 6 injections over 12 days ip(10ug of Derp2+Alum)

(group B)

ip(10ug of Derp2+Alum) ip(10ug of Derp2+Alum) sc(50ug of Derp2+40ug of Fve) 6 injections over 12 days ip(10ug of Derp2+Alum)

FIGURE 13

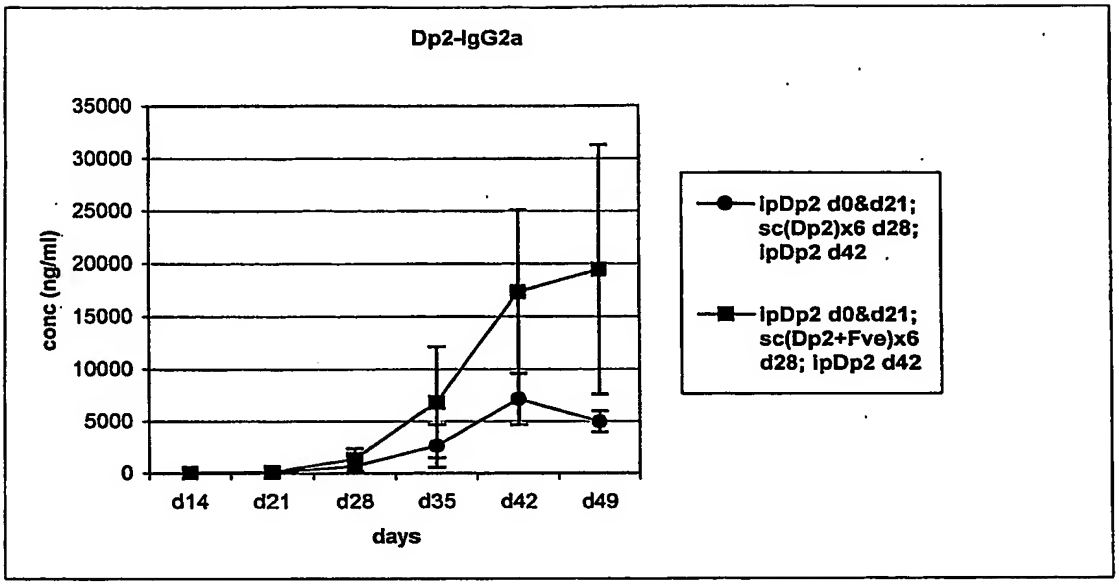
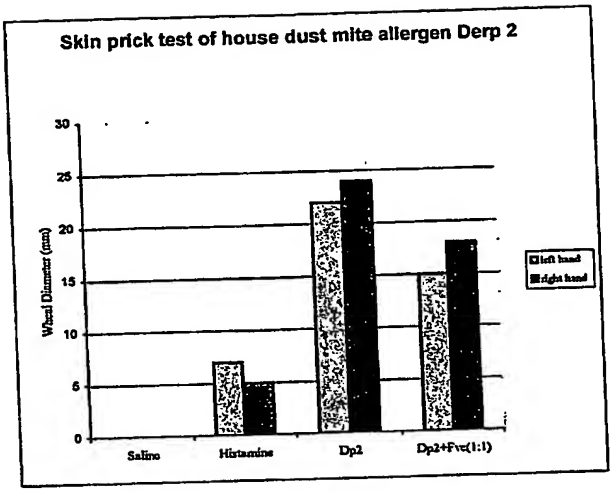

Dp2-IgG2a
35000
30000
25000
20000
15000
10000
5000
0
conc (ng/ml)
d14
d21
d28
d35
d42
d49
days
IpDp2 d0&d21; sc(Dp2)x6 d28; IpDp2 d42
IpDp2 d0&d21; sc(Dp2+Fve)x6 d28; IpDp2 d42

FIGURE 14

(A)


Skin prick test of house dust mite allergen Derp 2
Wheal Diameter (mm)
30
25
20
15
10
5
0
Saline
Histamine
Dp2
Dp2+Fve(1:1)
left hand
right hand

(B)

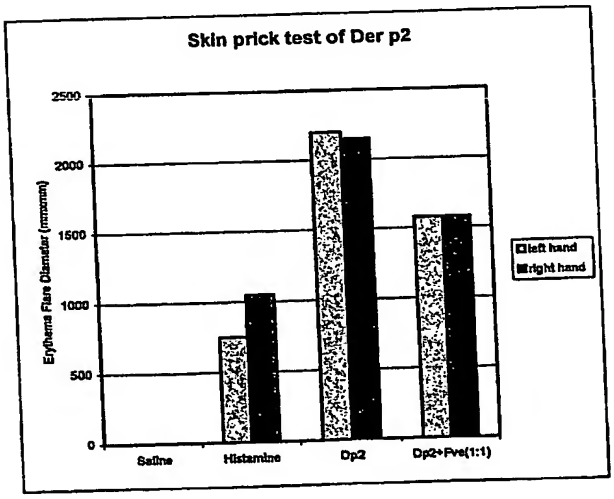

Skin prick test of Der p2
Erythema Flare Diameter (mmxmm)
2500
2000
1500
1000
500
0
Saline
Histamine
Dp2
Dp2+Fve(1:1)
left hand
right hand

FIGURE 15

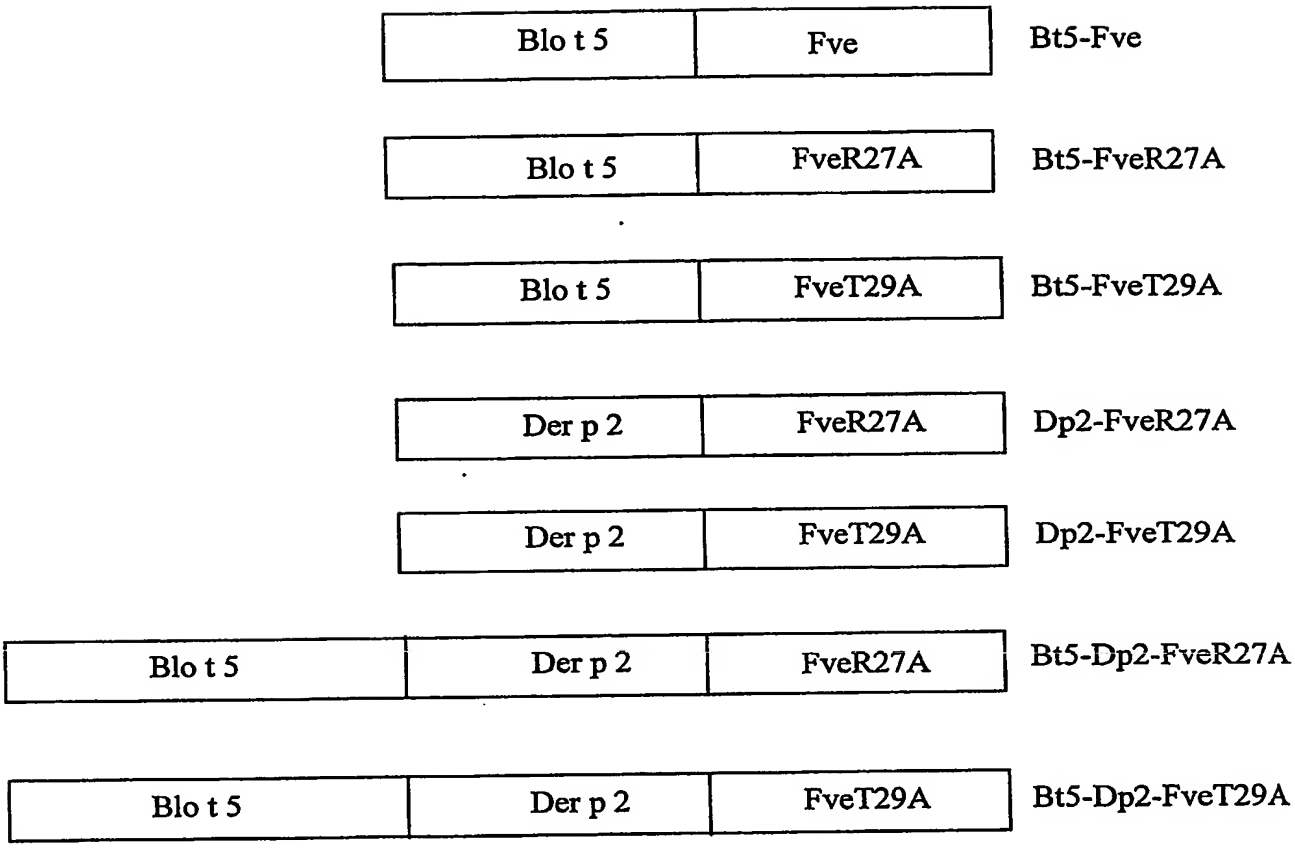

Blo t 5
Fve
Bt5-Fve
Blo t 5
FveR27A
Bt5-FveR27A
Blo t 5
FveT29A
Bt5-FveT29A
Der p 2
FveR27A
Dp2-FveR27A
Der p 2
FveT29A
Dp2-FveT29A
Blo t 5
Der p 2
FveR27A
Bt5-Dp2-FveR27A
Blo t 5
Der p 2
FveT29A
Bt5-Dp2-FveT29A

FIGURE 16

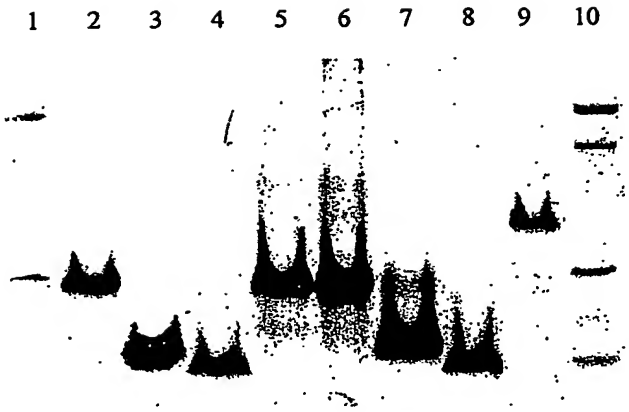
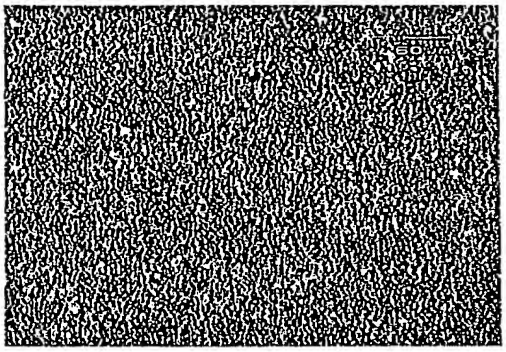


FIGURE 17

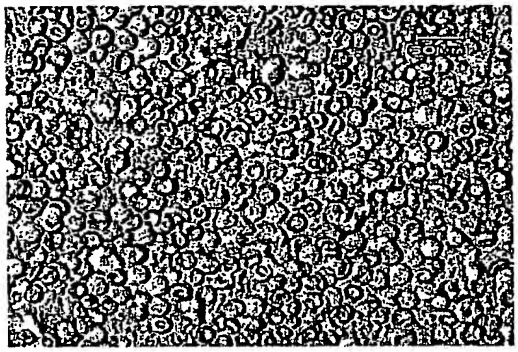
(1a)

Control: Non-stimulated (10x10 magnification)



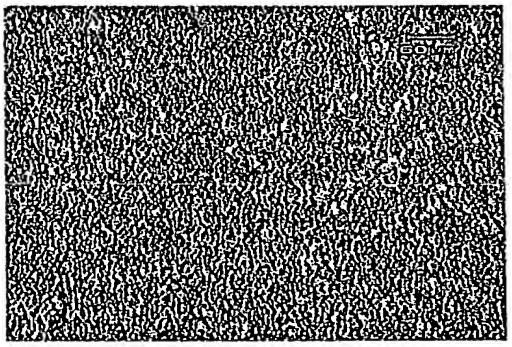
(1b)

Control : Non-stimulated (40x10 magnification)



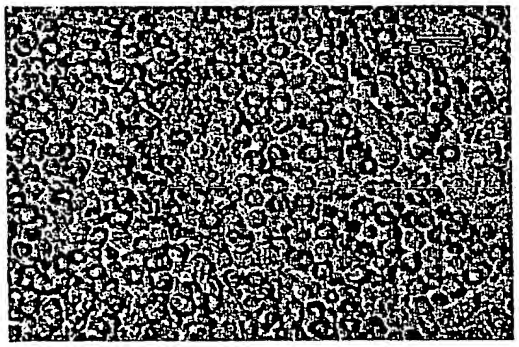
(2a)

20ug of GST 10x10



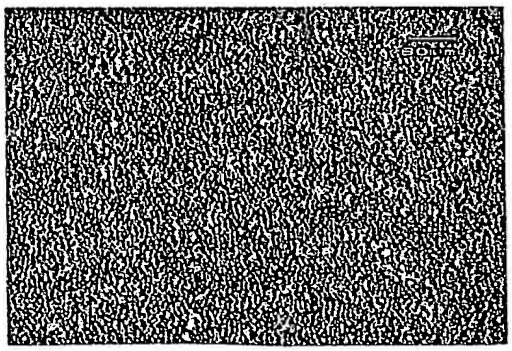
(2b)

20ug of GST 40x10



(3a)

20ug of Blo t 5 10x10



(3b)

20ug of Blo t 5 40x10

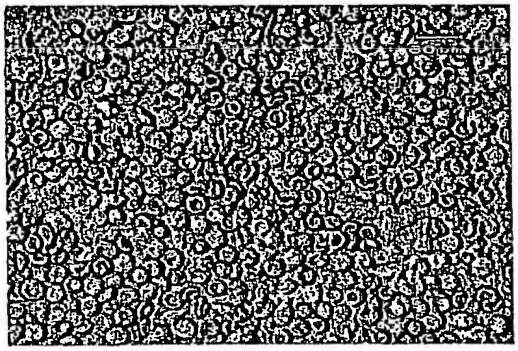
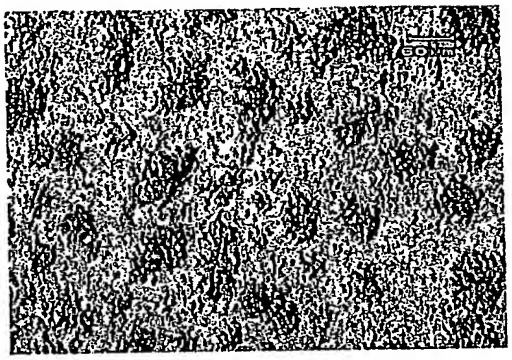


FIGURE 18

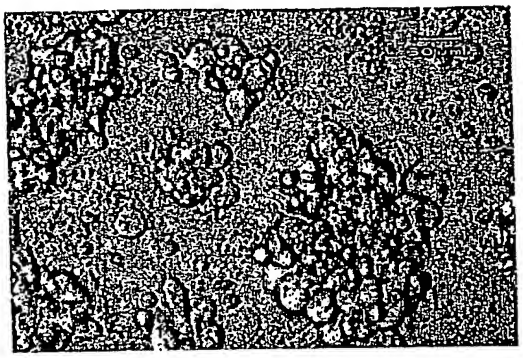
(4a)

20ug of native FIP-Fve 10x10



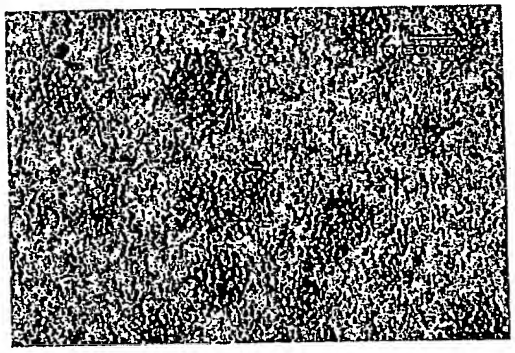
(4b)

20ug of native FIP-Fve 40x10



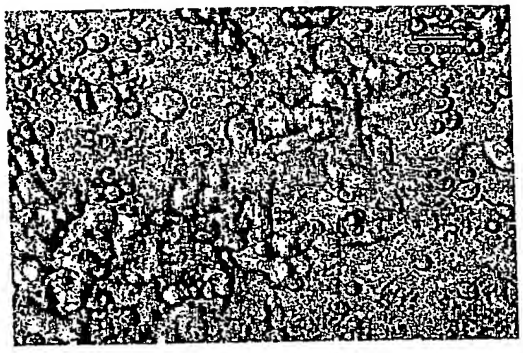
(5a)

20ug of Bt5-Fve 10x10



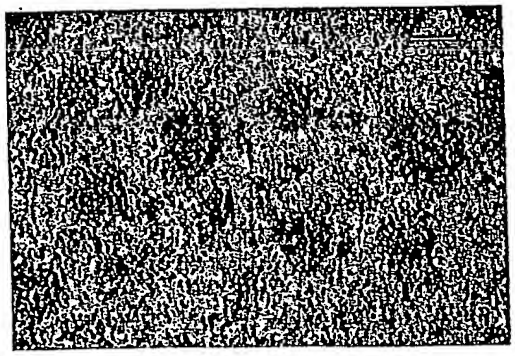
(5b)

20ug of Bt5-Fve 40x10



(6a)

40ug of Bt5-Fve 10x10



(6b)

40ug of Bt5-Fve 40x10

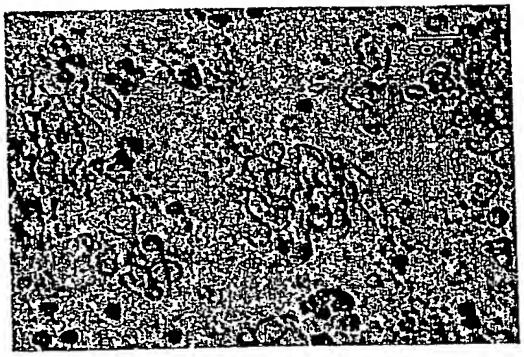
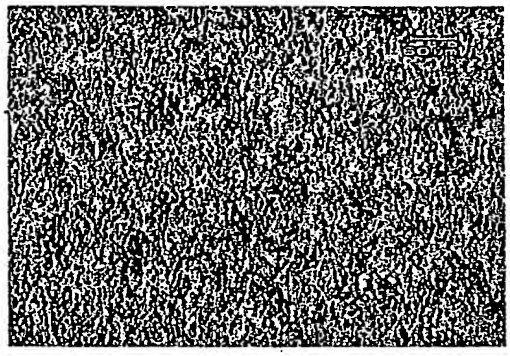


FIGURE 18 (CONTINUED)

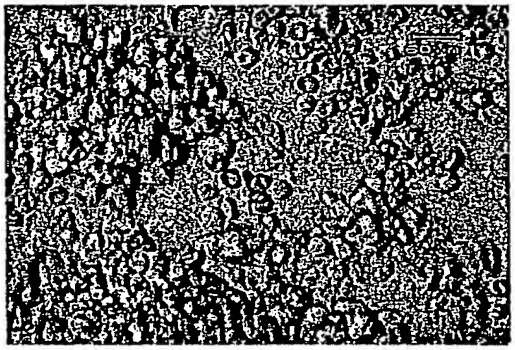
(7a)

40ug of Bt5-FveR27A 10x10



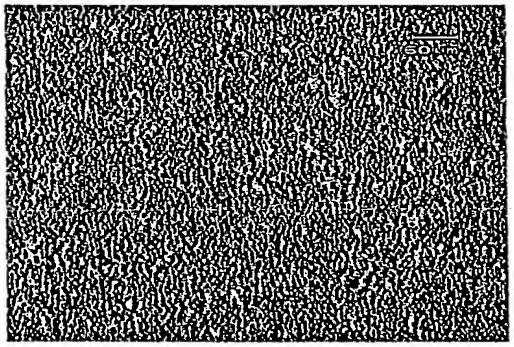
(7b)

40ug of Bt5-FveR27A 40x10



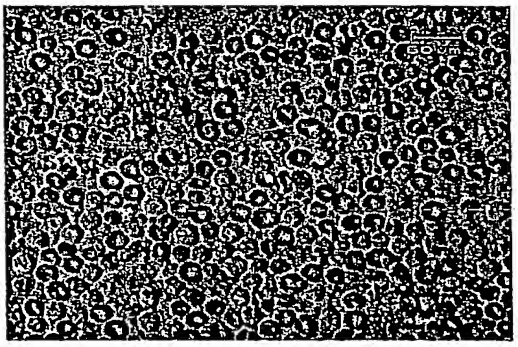
(8a)

20ug of Der p 2 10x10



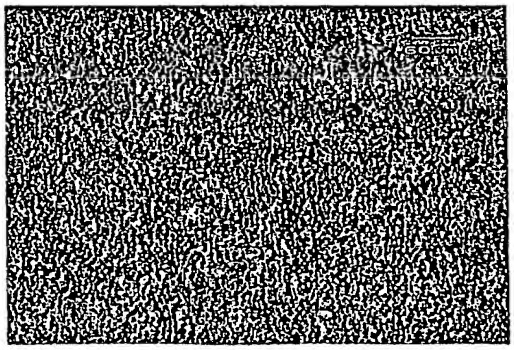
(8b)

20ug of Der p 2 40x10



(9a)

40ug of GST-Dp2-FveR27A 10x10



(9b)

40ug of GST-Dp2-FveR27A 40x10

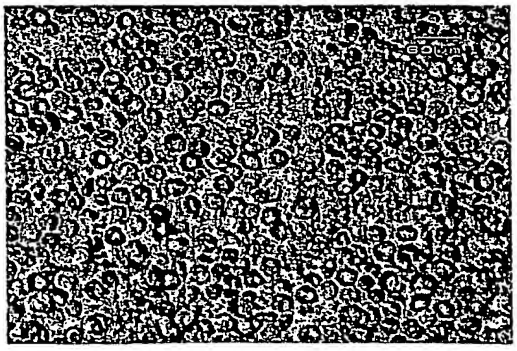
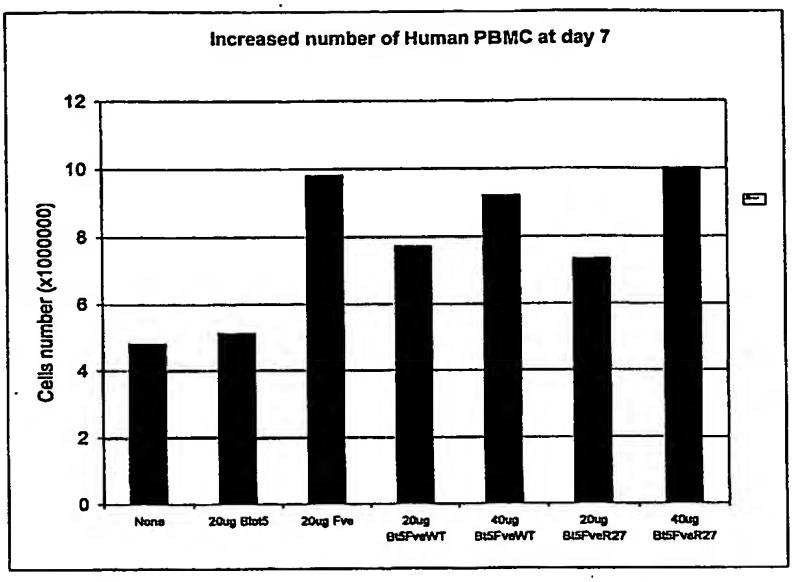


FIGURE 18 (CONTINUED)

(A)


Increased number of Human PBMC at day 7
Cells number (x1000000)
12
10
8
6
4
2
0
None
20ug Btbt5
20ug Fve
20ug BtSFveWT
40ug BtSFveWT
20ug BtSFveR27
40ug BtSFveR27

(B)

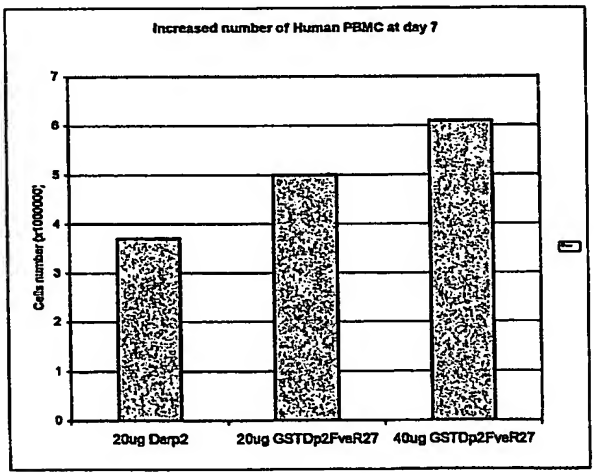

Increased number of Human PBMC at day 7
Cells number (x1000000)
7
6
5
4
3
2
1
0
20ug Darp2
20ug GSTDp2FveR27
40ug GSTDp2FveR27

FIGURE 19

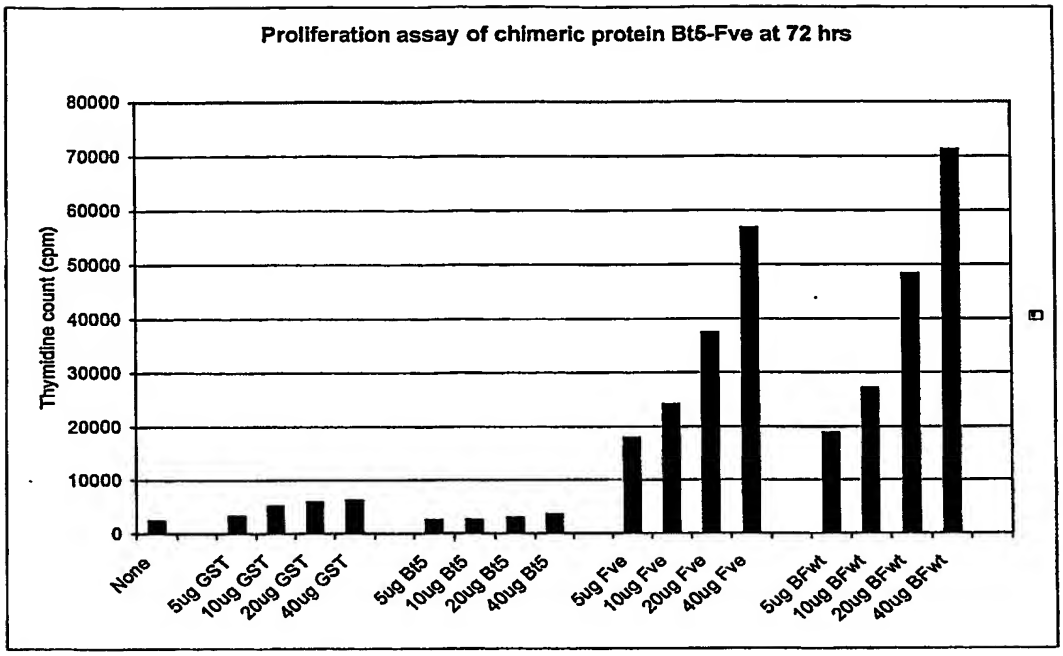

Proliferation assay of chimeric protein Bt5-Fve at 72 hrs
Thymidine count (cpm)
80000
70000
60000
50000
40000
30000
20000
10000
0
None
5ug GST
10ug GST
20ug GST
40ug GST
5ug Bt5
10ug Bt5
20ug Bt5
40ug Bt5
5ug Fve
10ug Fve
20ug Fve
40ug Fve
5ug BFwt
10ug BFwt
20ug BFwt
40ug BFwt

FIGURE 20

FIGURE 21

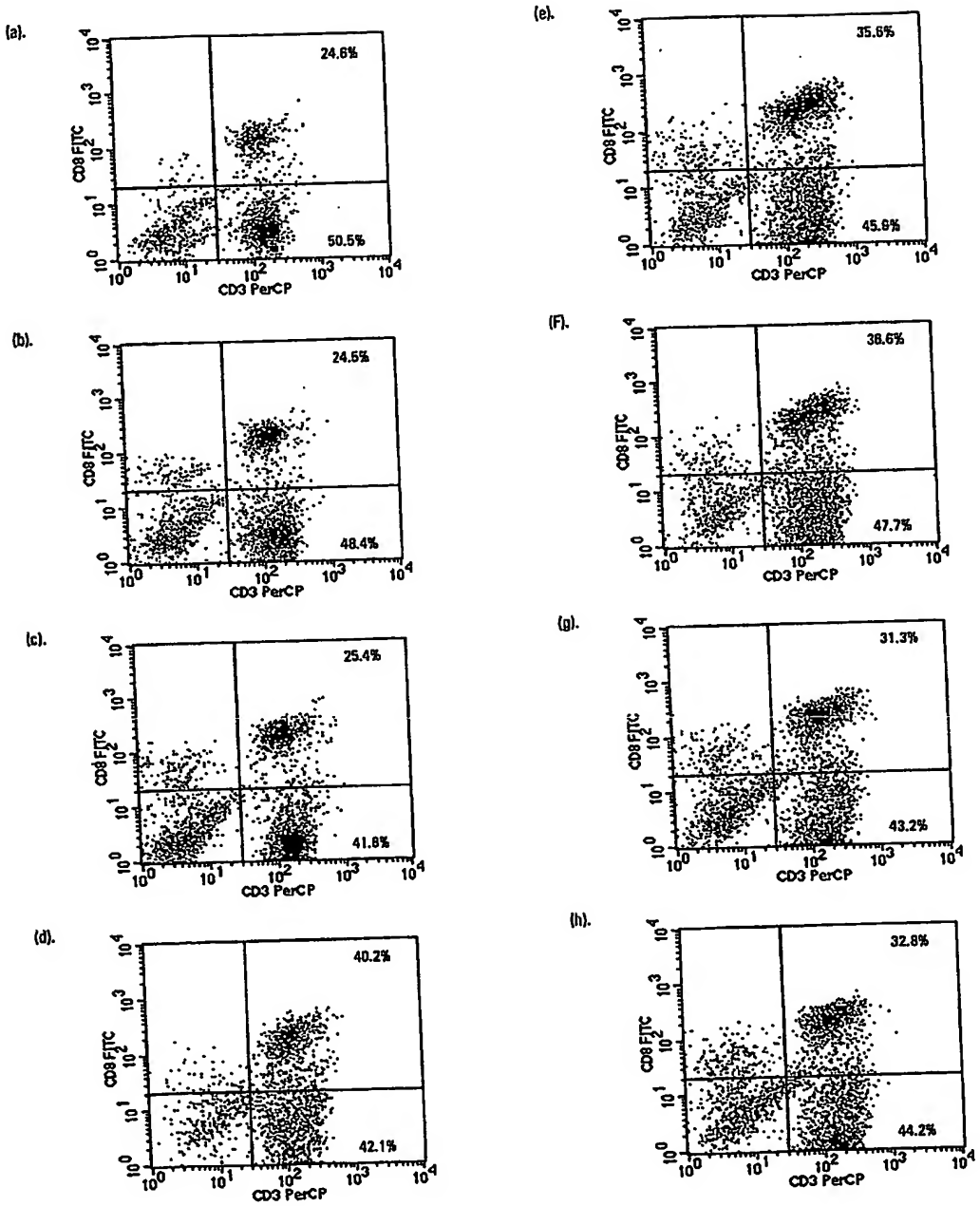

(a).
24.6%
50.5%
(b).
24.5%
48.4%
(c).
25.4%
41.8%
(d).
40.2%
42.1%
(e).
35.6%
45.6%
(F).
38.6%
47.7%
(g).
31.3%
43.2%
(h).
32.8%
44.2%
CD8 FITC
CD3 PerCP

FIGURE 22A

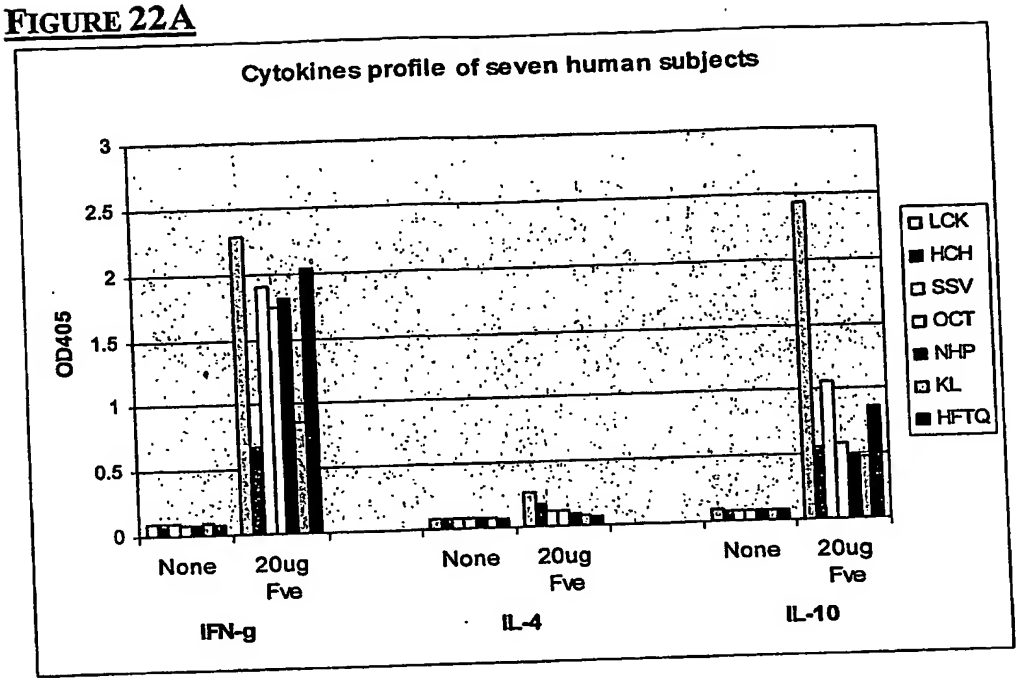
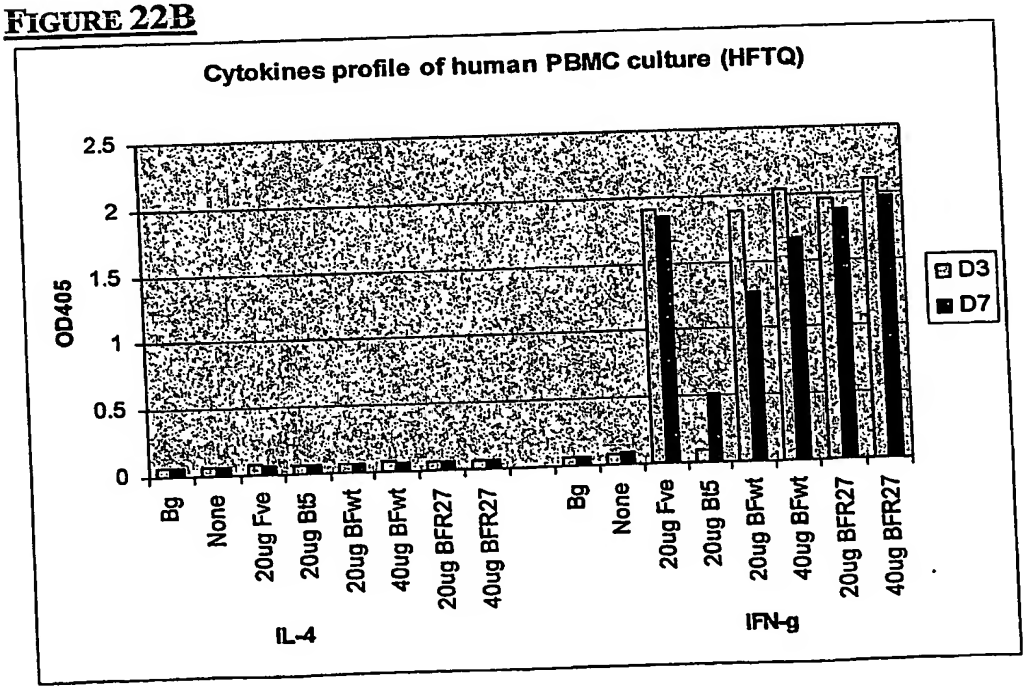


FIGURE 22B



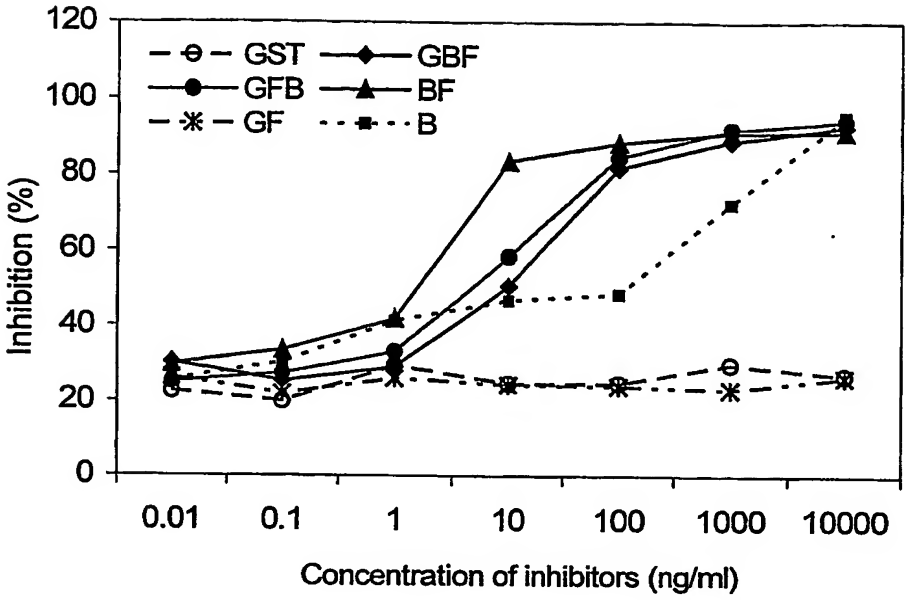
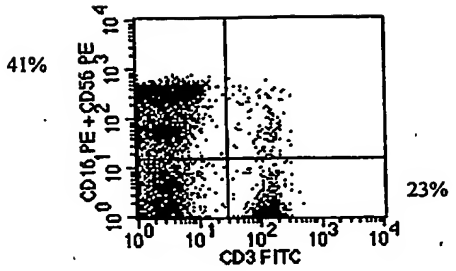
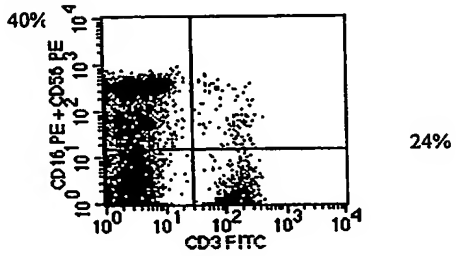

120
100
80
60
40
20
0
Inhibition (%)
GST
GBF
GFB
BF
GF
B
0.01
0.1
1
10
100
1000
10000
Concentration of inhibitors (ng/ml)

FIGURE 23

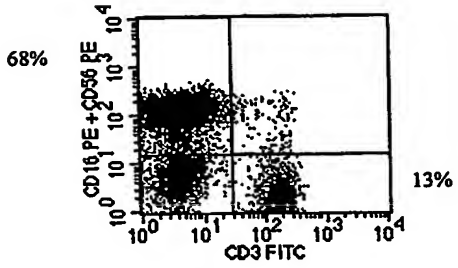
(a).



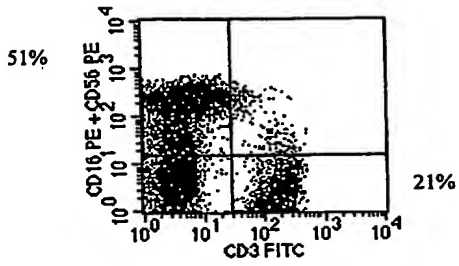
(b).



(c).



(d).



(e).

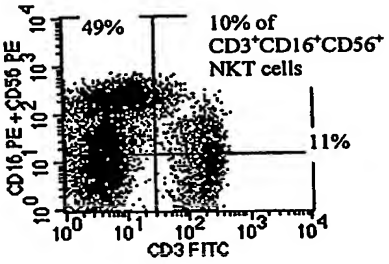
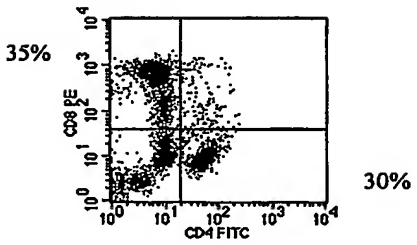
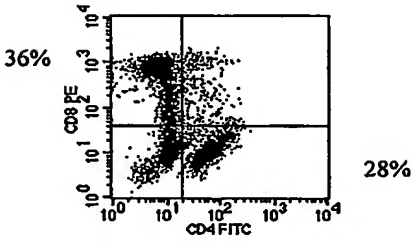


FIGURE 24

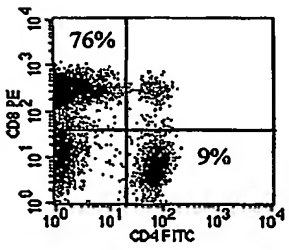
(a).



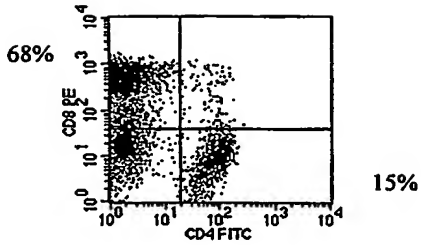
(b).



(c).



(d).



(e).

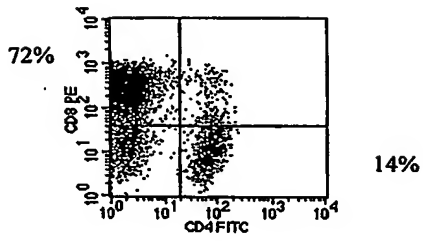
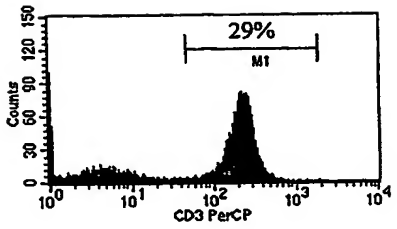
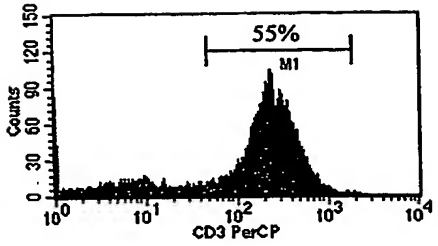


FIGURE 25

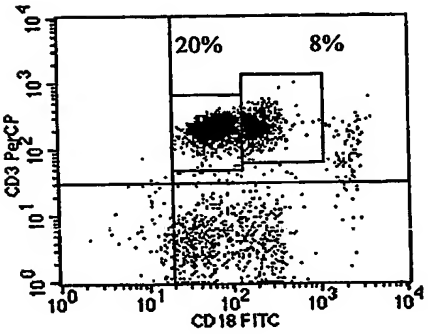
(a).



(b).



(c).



(d).

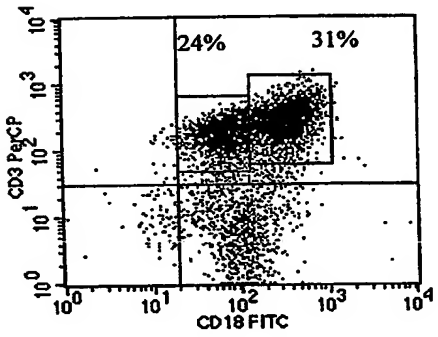
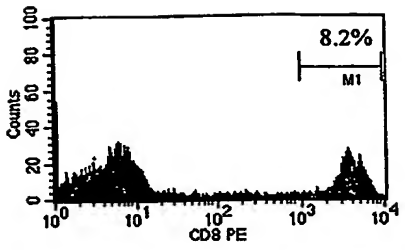
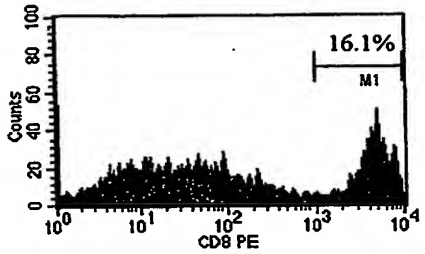


FIGURE 26

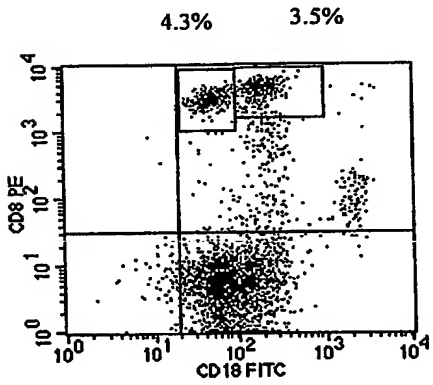
(a).



(b).



(c).



(d).

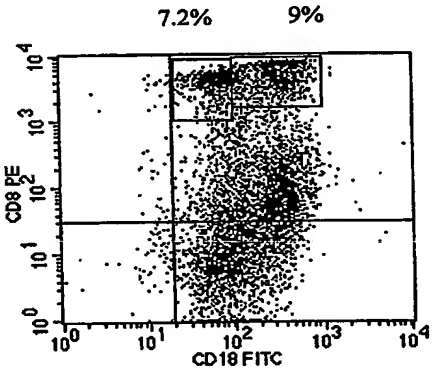


FIGURE 27

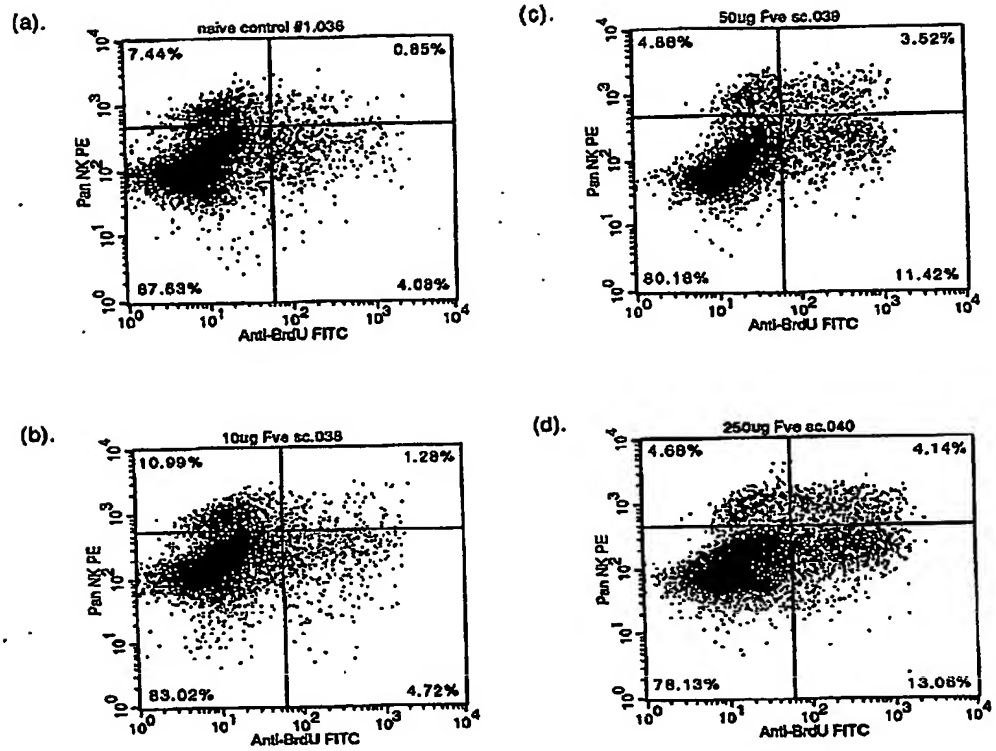
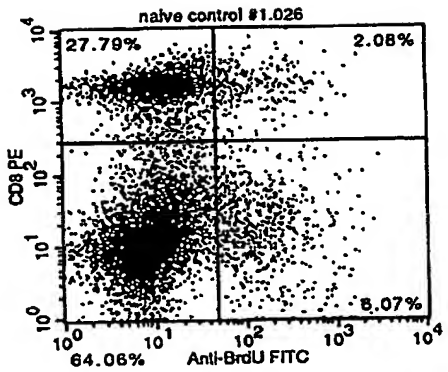
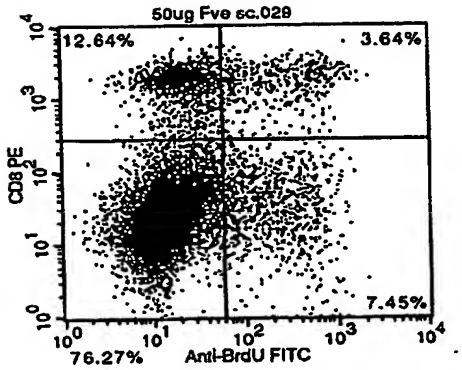

(a).
naive control #1.036
7.44%
0.85%
87.63%
4.08%
Pan NK PE
Anti-BrdU FITC
(c).
50ug Fve sc.039
4.88%
3.52%
80.18%
11.42%
Pan NK PE
Anti-BrdU FITC
(b).
10ug Fve sc.038
10.99%
1.28%
83.02%
4.72%
Pan NK PE
Anti-BrdU FITC
(d).
250ug Fve sc.040
4.68%
4.14%
78.13%
13.06%
Pan NK PE
Anti-BrdU FITC

FIGURE 28

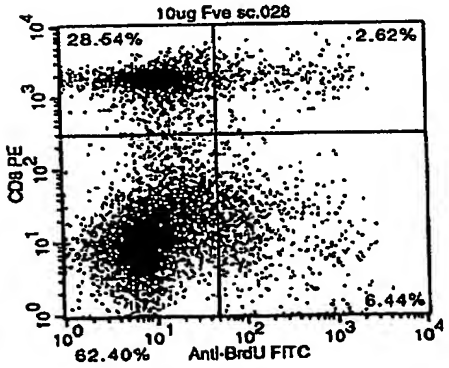
(a).



(c).



(b).



(d).

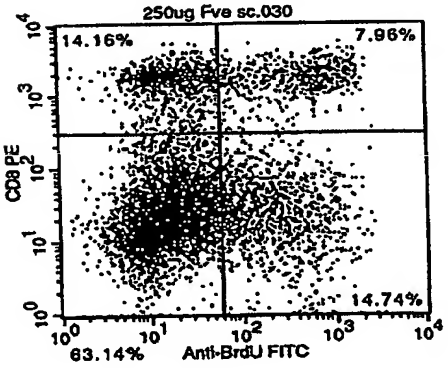


FIGURE 29

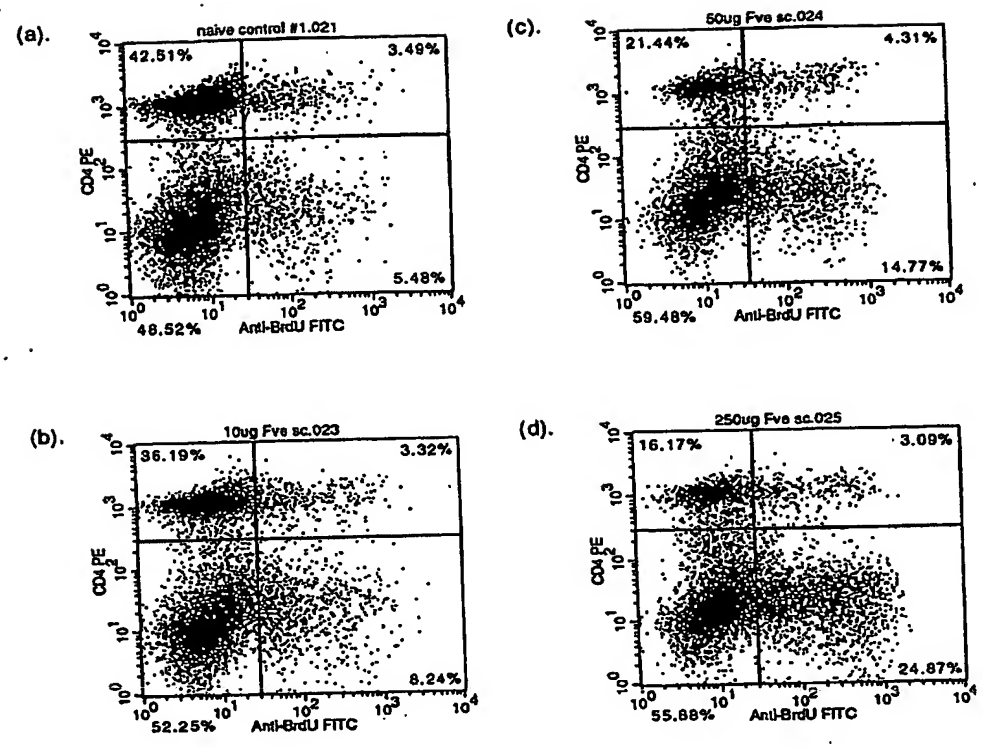

(a).
naive control #1.021
42.51%
3.49%
5.48%
48.52%
CD4 PE
Anti-BrdU FITC
(c).
50ug Fve sc.024
21.44%
4.31%
14.77%
59.48%
CD4 PE
Anti-BrdU FITC
(b).
10ug Fve sc.023
36.19%
3.32%
8.24%
52.25%
CD4 PE
Anti-BrdU FITC
(d).
250ug Fve sc.025
16.17%
3.09%
24.87%
55.88%
CD4 PE
Anti-BrdU FITC

FIGURE 30

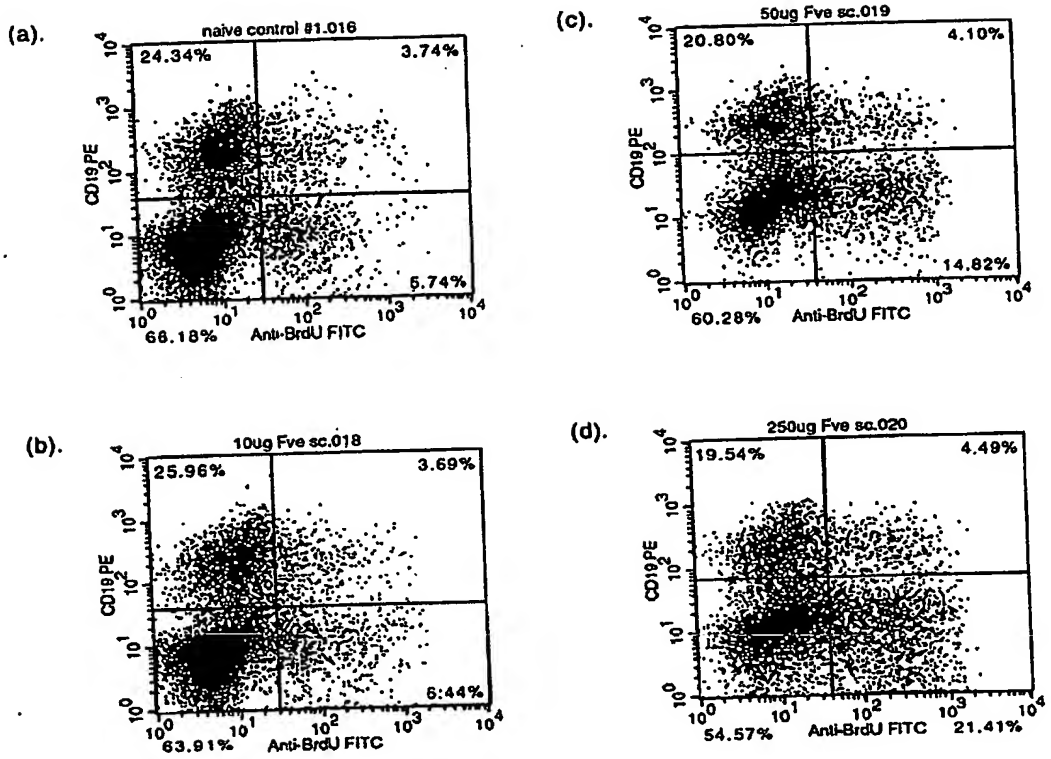

(a).
naive control #1.016
24.34%
3.74%
5.74%
66.18%
CD19 PE
Anti-BrdU FITC
(b).
10ug Fve sc.018
25.96%
3.69%
6.44%
63.91%
CD19 PE
Anti-BrdU FITC
(c).
50ug Fve sc.019
20.80%
4.10%
14.82%
60.28%
CD19 PE
Anti-BrdU FITC
(d).
250ug Fve sc.020
19.54%
4.49%
54.57%
21.41%
CD19 PE
Anti-BrdU FITC

FIGURE 31

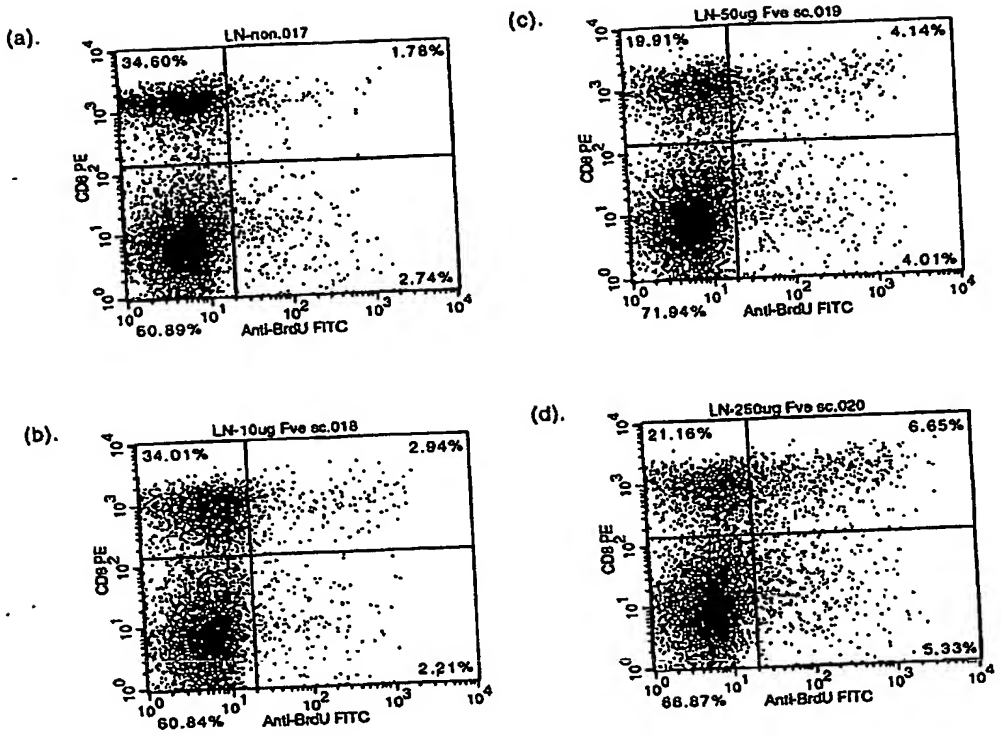
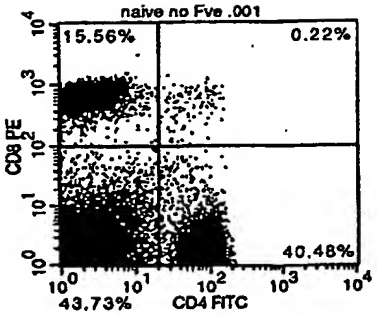
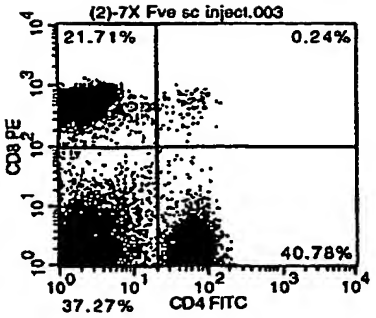

(a).
LN-non.017
34.60%
1.78%
2.74%
60.89%
CD8 PE
Anti-BrdU FITC
(b).
LN-10ug Fve sc.018
34.01%
2.94%
2.21%
60.84%
CD8 PE
Anti-BrdU FITC
(c).
LN-50ug Fve sc.019
19.91%
4.14%
4.01%
71.94%
CD8 PE
Anti-BrdU FITC
(d).
LN-250ug Fve sc.020
21.16%
6.65%
5.33%
66.87%
CD8 PE
Anti-BrdU FITC

FIGURE 32

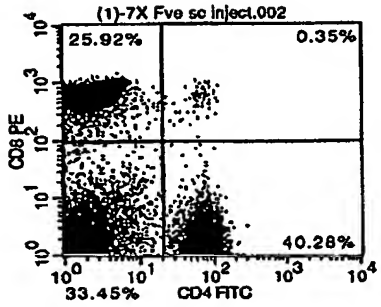
(a).



(c).



(b).



(d).

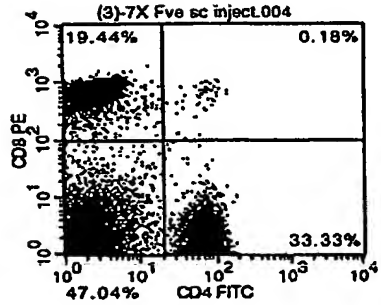
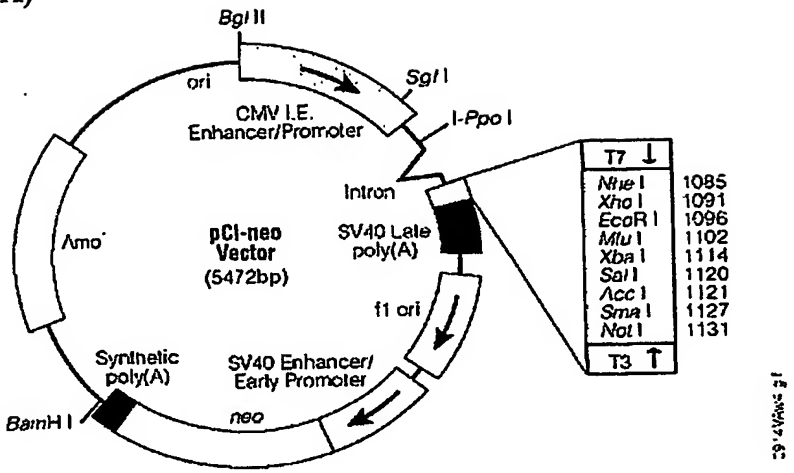


FIGURE 33

(A)


BglII
ori
SgfI
CMV I.E.
Enhancer/Promoter
I-PpoI
Intron
T7
NheI 1085
XhoI 1091
EcoRI 1096
MluI 1102
XbaI 1114
SalI 1120
AccI 1121
SmaI 1127
NotI 1131
T3
Amp
pCI-neo
Vector
(5472bp)
SV40 Late
poly(A)
f1 ori
Synthetic
poly(A)
SV40 Enhancer/
Early Promoter
BamHI
neo

(B)

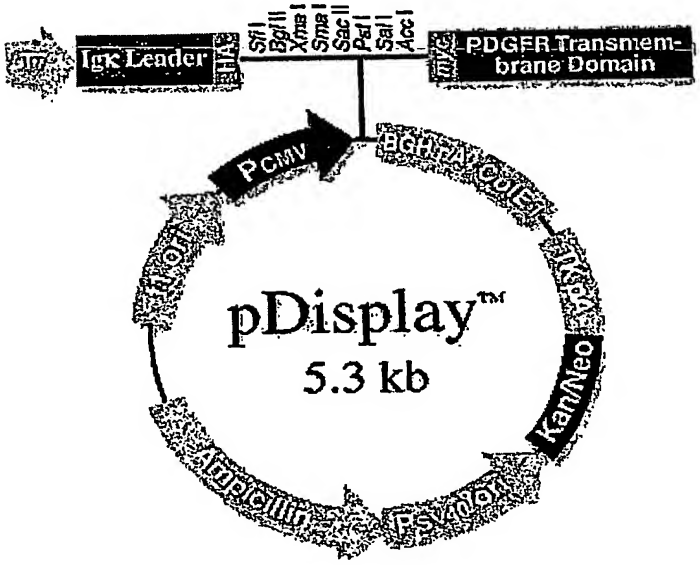

Igκ Leader
SfiI
BglII
XmaI
SmaI
SacII
PstI
SalI
AccI
PDGFR Transmembrane Domain
PCMV
BGH pA
pDisplay™
5.3 kb
Kan/Neo
pUC ori
f1 ori
SV40 pA

FIGURE 34

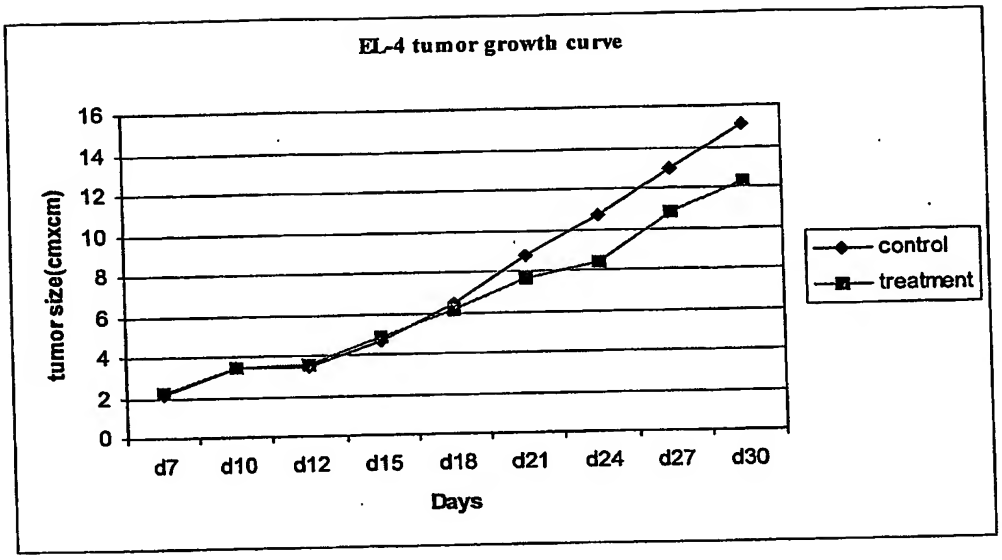

EL-4 tumor growth curve
tumor size(cmxcm)
16
14
12
10
8
6
4
2
0
d7
d10
d12
d15
d18
d21
d24
d27
d30
Days
control
treatment

FIGURE 35

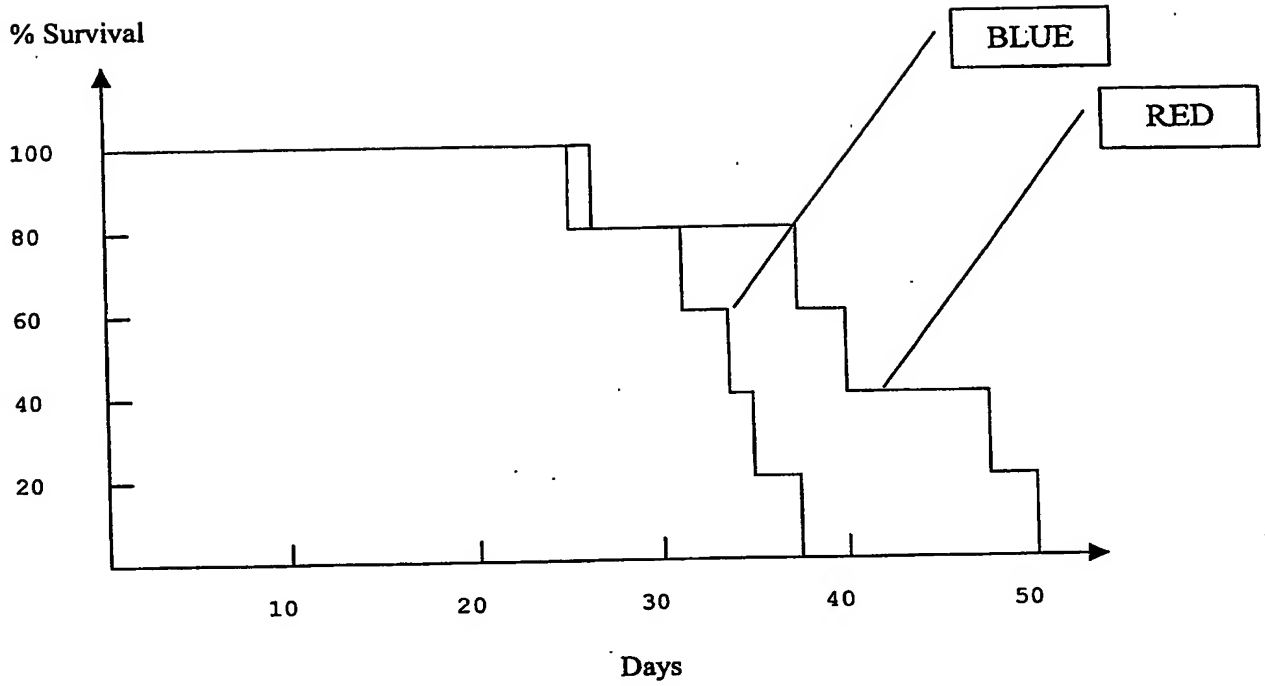


FIGURE 36

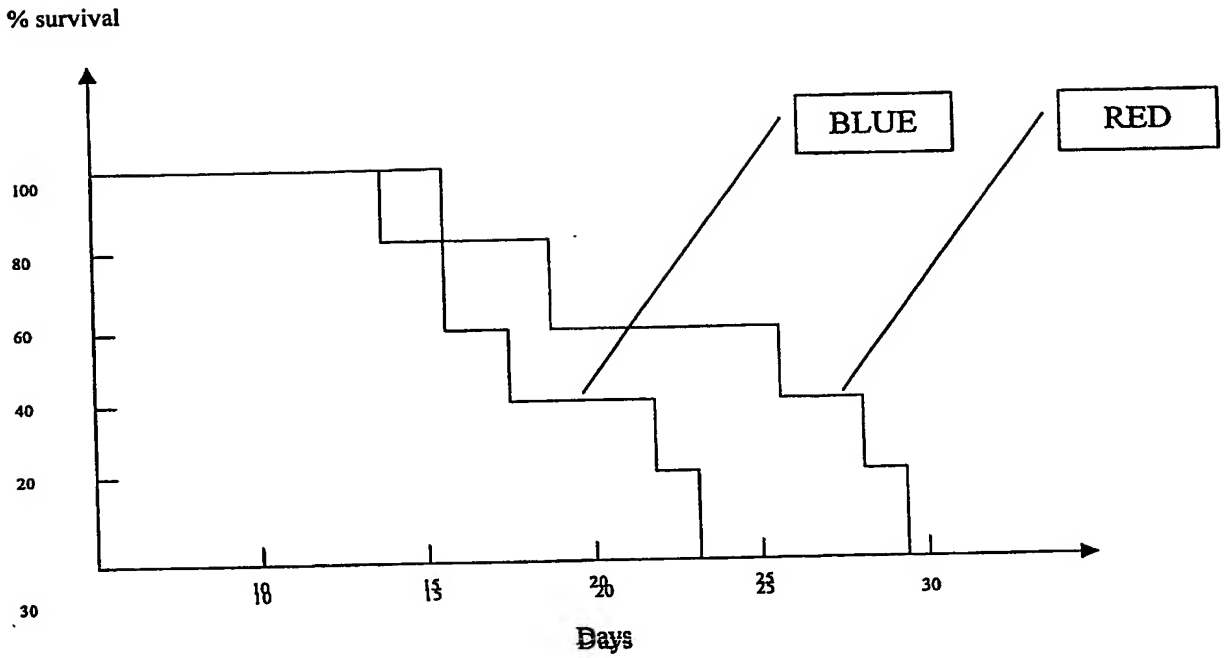


FIGURE 37

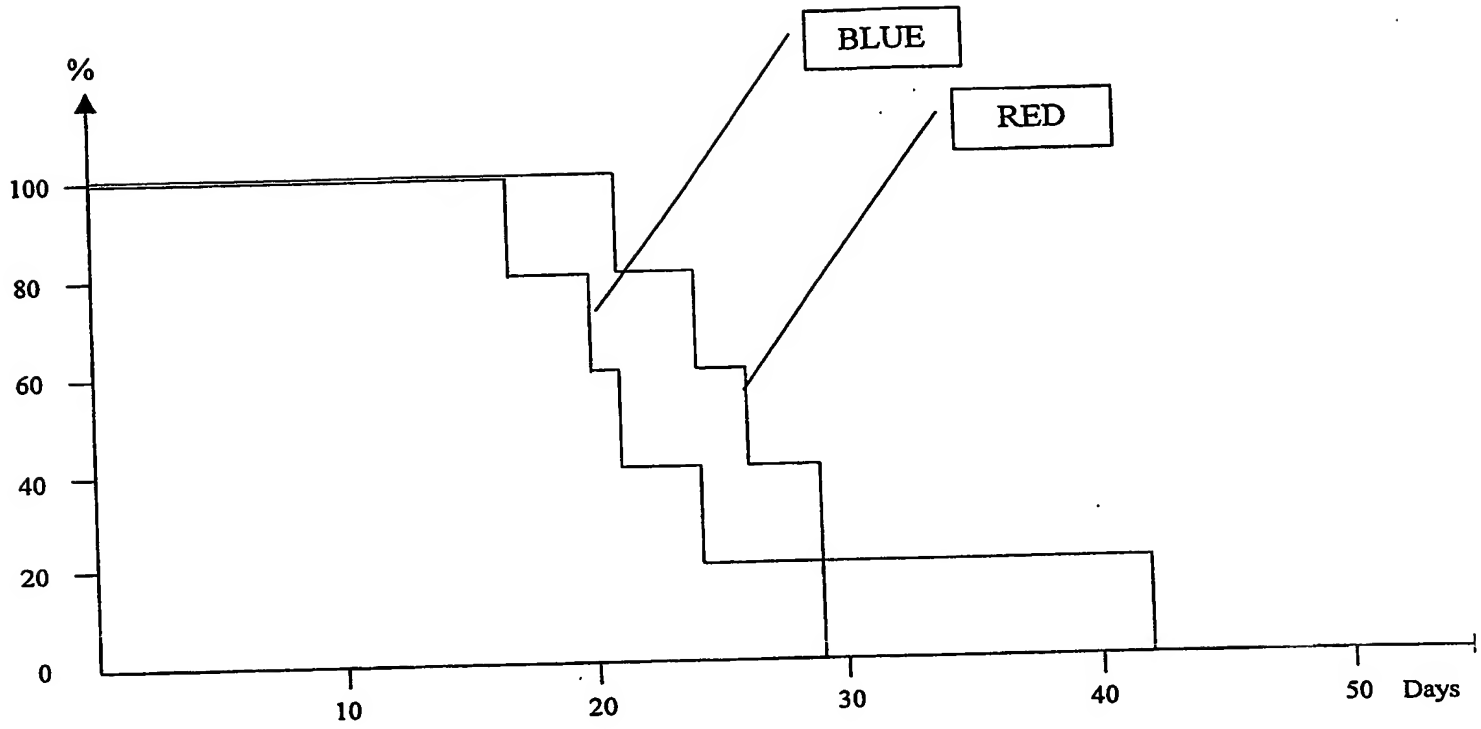


FIGURE 38

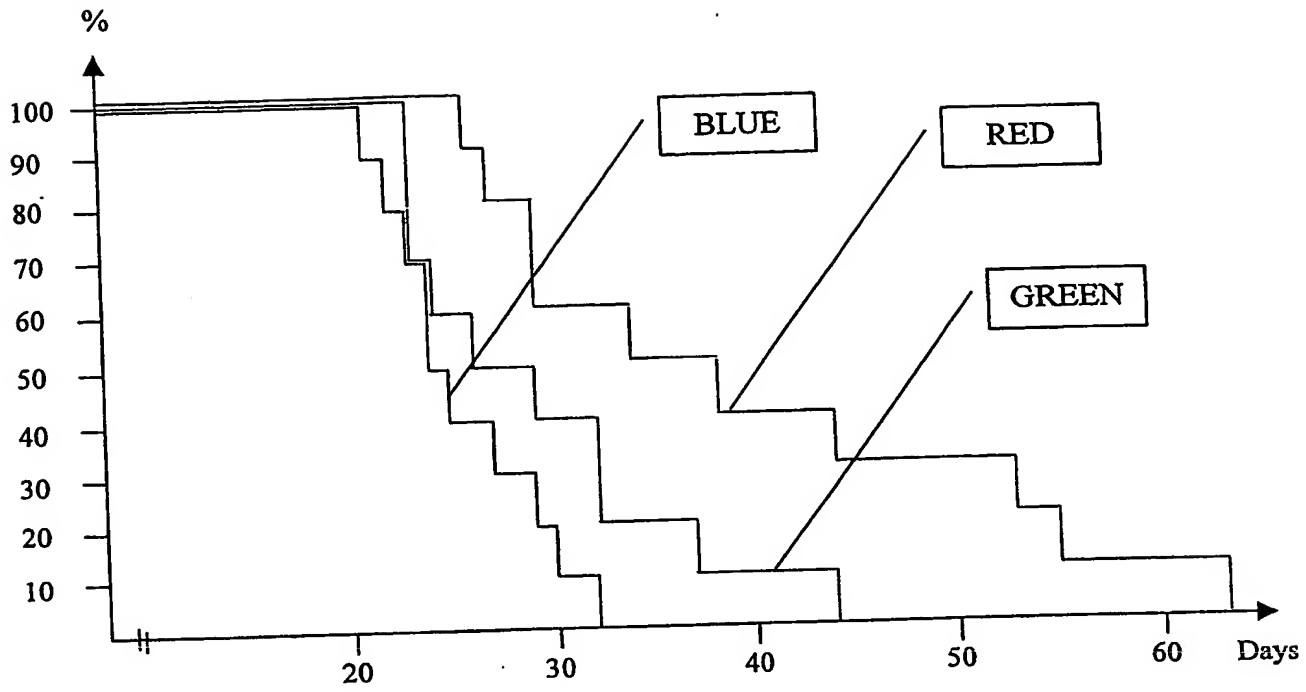


FIGURE 39

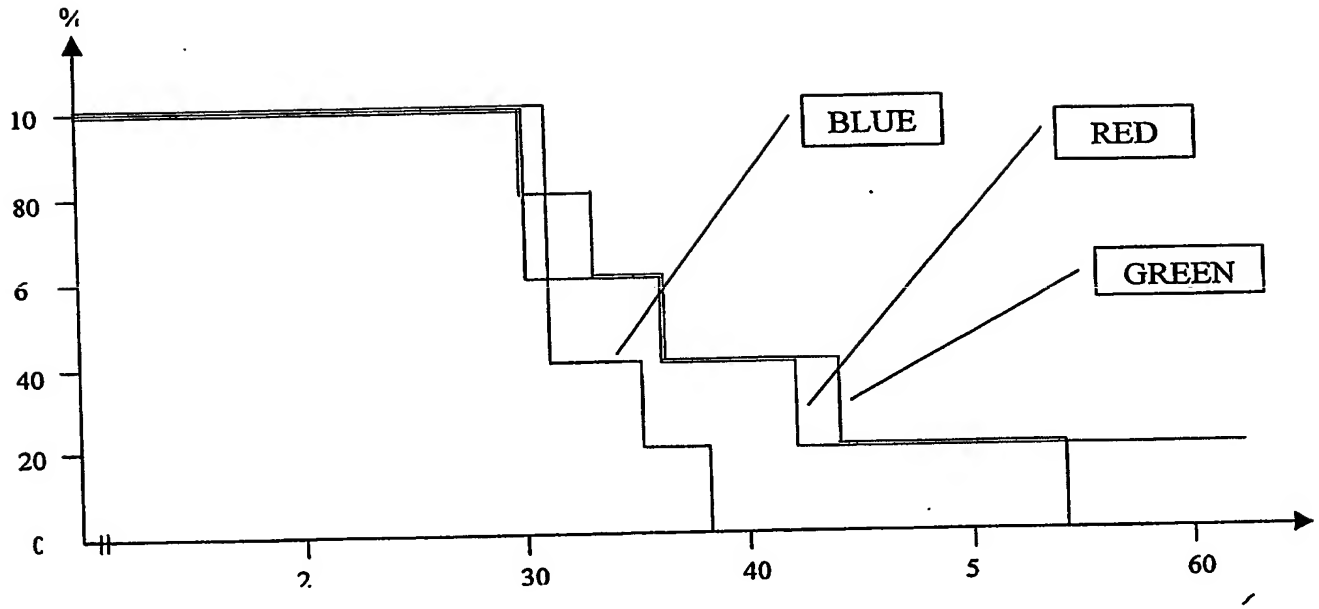
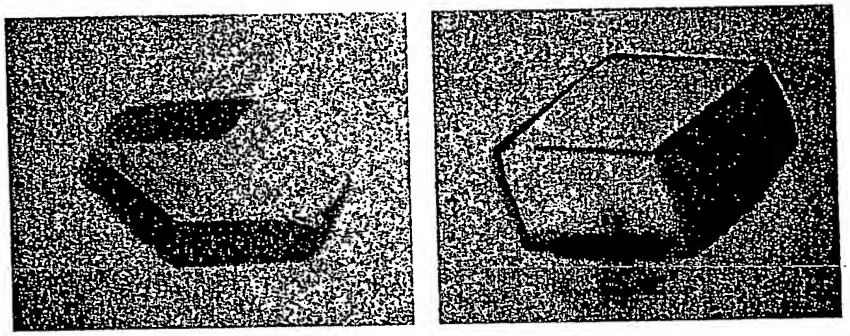


FIGURE 40

FIGURE 41



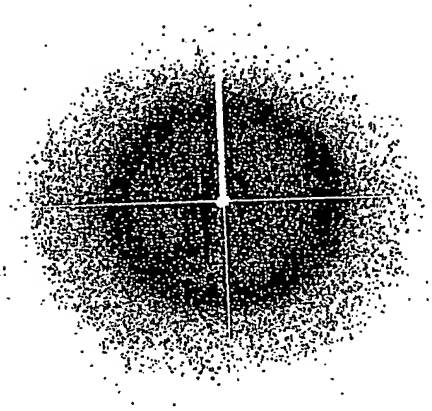
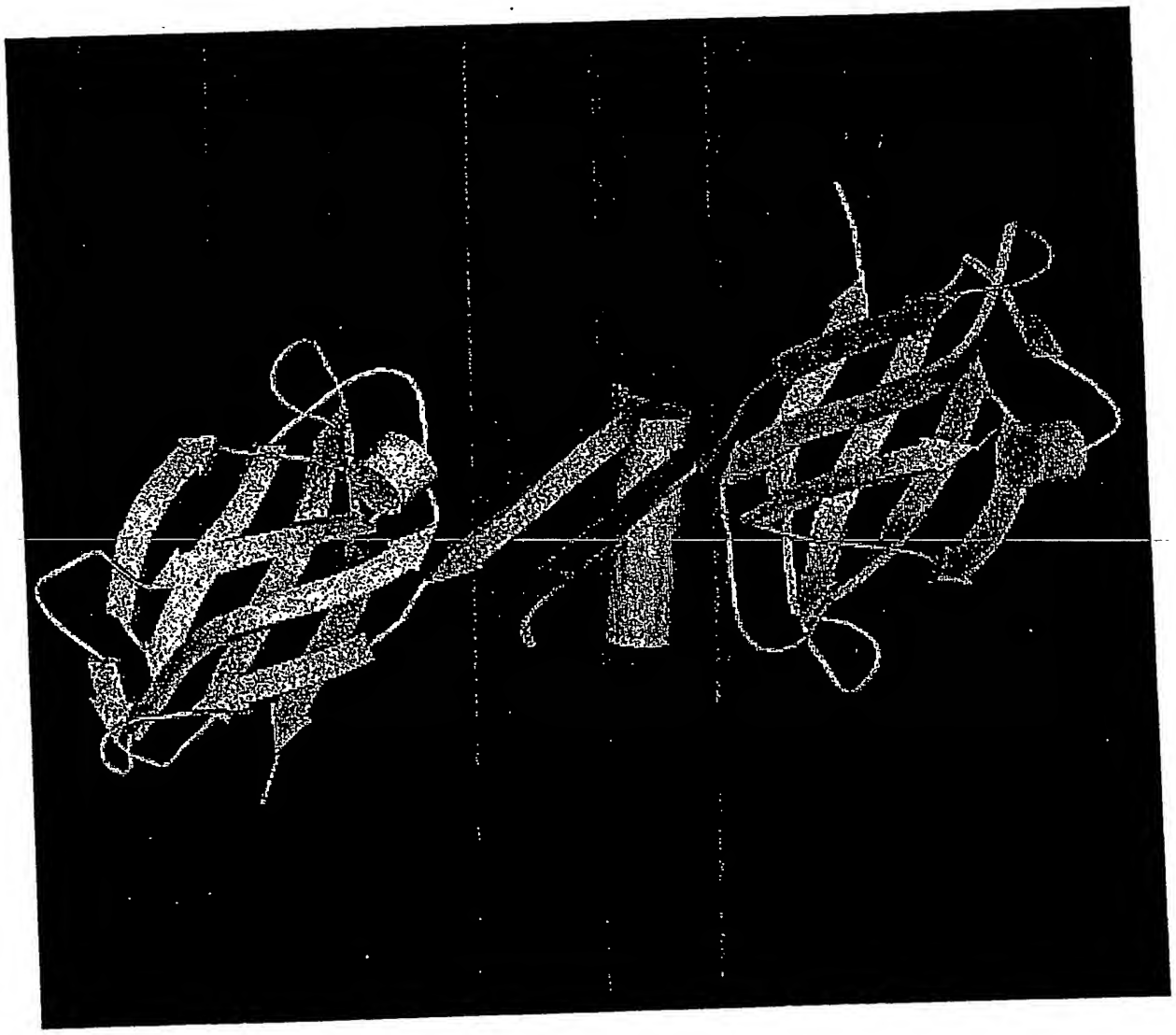


FIGURE 42

FIGURE 43



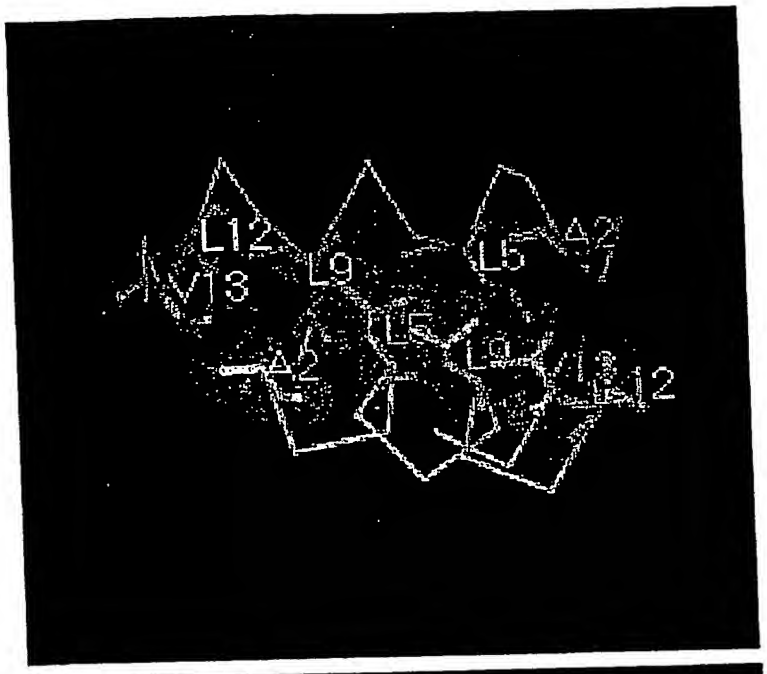


FIGURE 44A

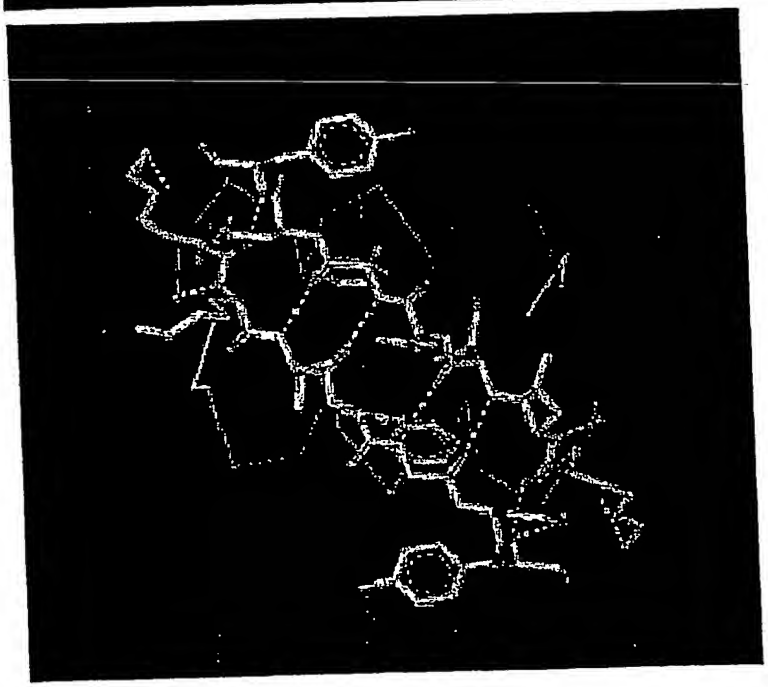
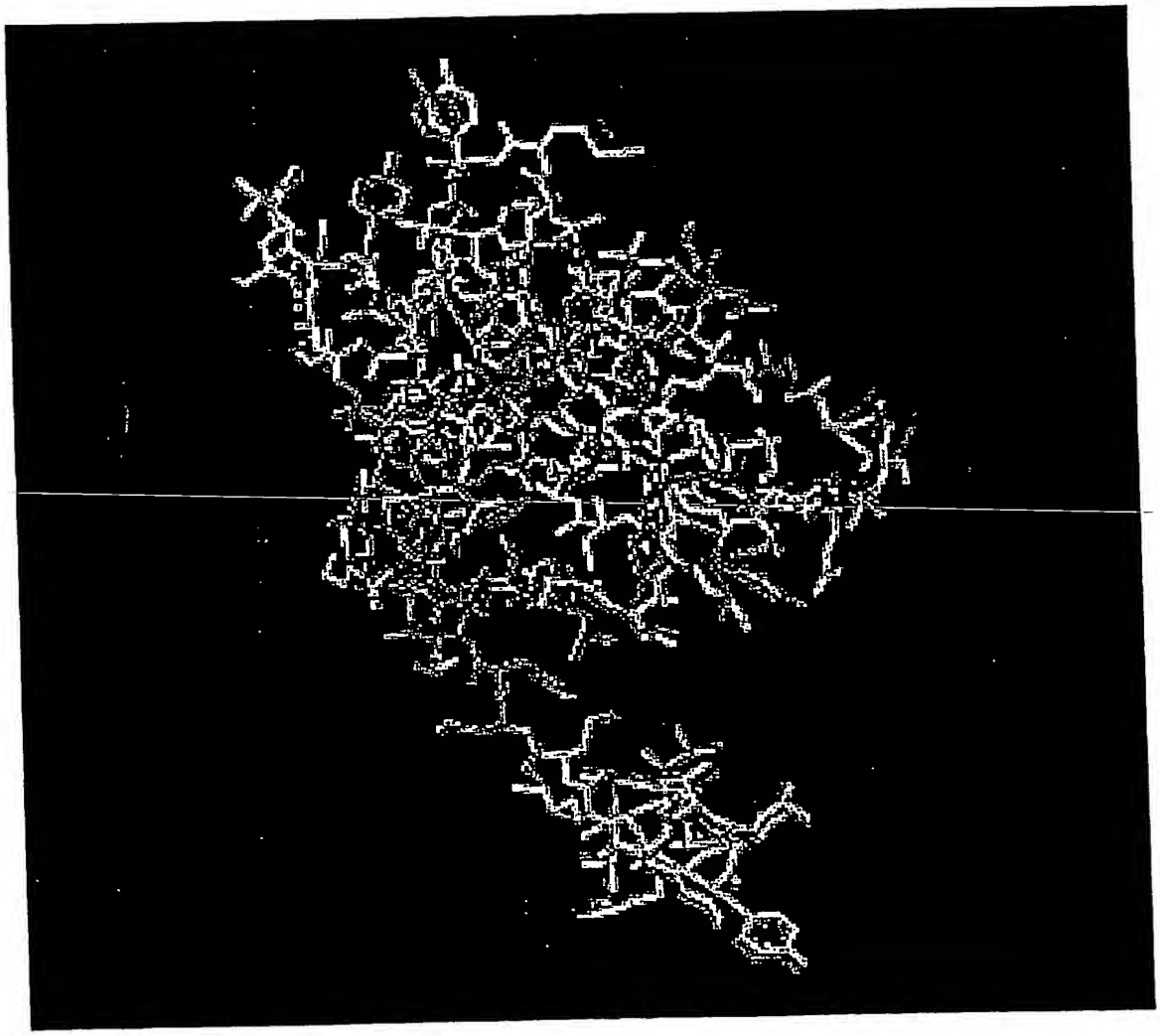


FIGURE 44B

FIGURE 44C



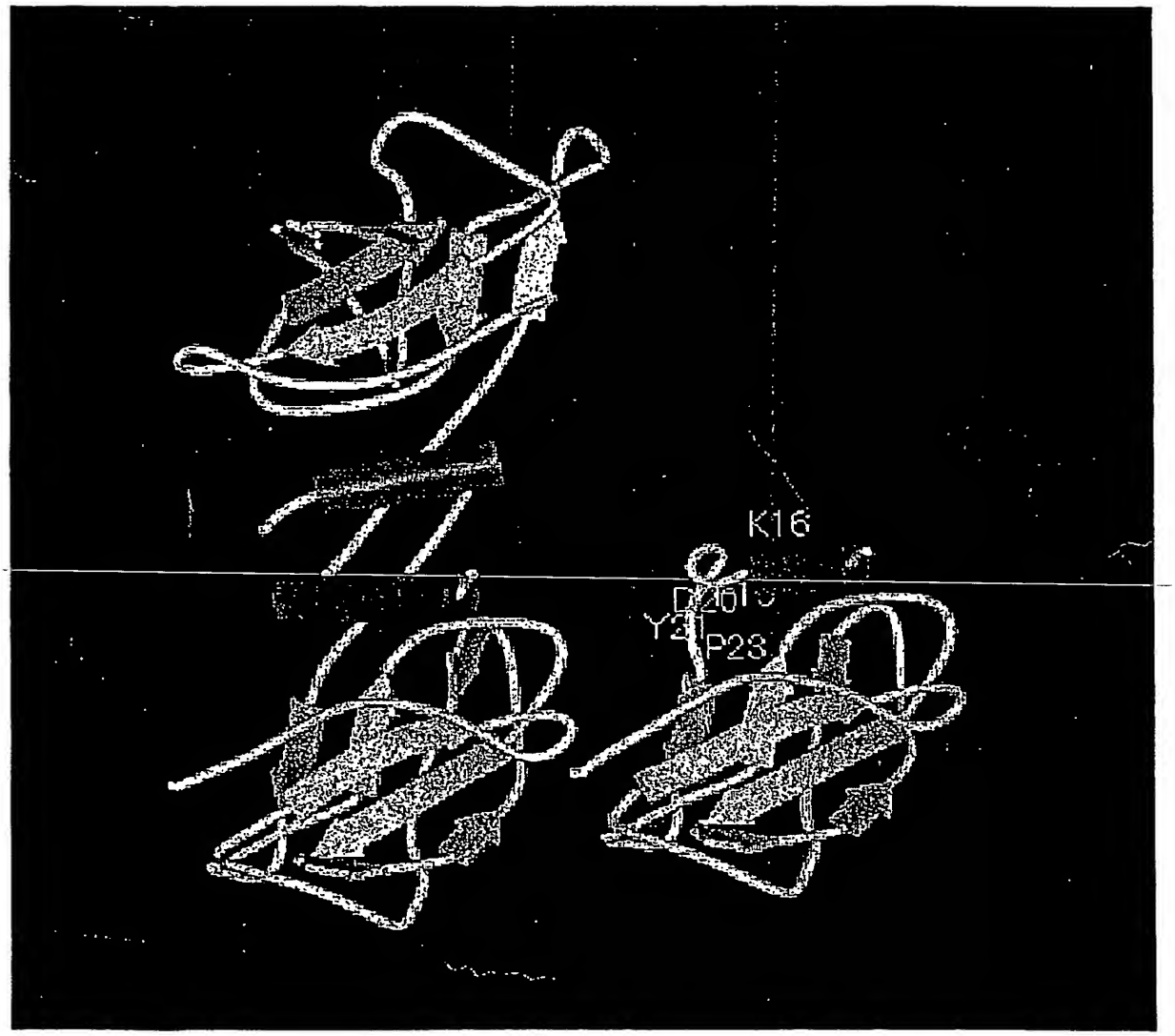


FIGURE 45A FIGURE 45B